

PLANT PARASITIC NEMATODES IN SUBTROPICAL AND TROPICAL AGRICULTURE

3rd Edition

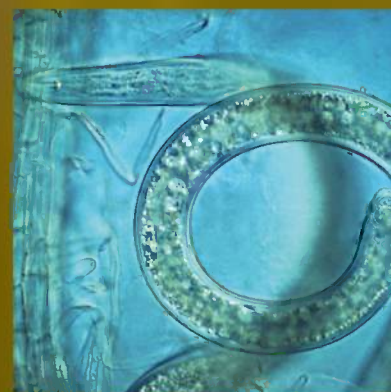
Edited by

Richard A. Sikora

Danny Coyne

Johannes Hallmann

Patricia Timper



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Contents

Contributors	vii
About the Editors	xi
Foreword	xvii
Acknowledgements	xix
Dedication	xxi
1 Reflections and Challenges: Nematology in Subtropical and Tropical Agriculture	1
<i>Richard A. Sikora, Danny Coyne, Johannes Hallmann and Patricia Timper</i>	
2 Identification, Morphology and Biology of Plant Parasitic Nematodes	20
<i>David J. Hunt, Juan E. Palomares-Rius and Rosa H. Manzanilla-López</i>	
3 Nematode Ecology and Soil Health	62
<i>Sara Sánchez-Moreno and Howard Ferris</i>	
4 Methods for Extraction, Processing and Detection of Plant and Soil Nematodes	87
<i>Johannes Hallmann and Sergei A. Subbotin</i>	
5 Nematode Parasites of Rice	120
<i>Deliang Peng, Hari S. Gaur and John Bridge</i>	
6 Nematode Parasites of Cereals	163
<i>Abdelfattah A. Dababat and Hendrika Fourie</i>	
7 Nematode Parasites of Potato and Sweet Potato	222
<i>Björn Niere and Hannah Wangari Karuri</i>	
8 Nematode Parasites of Tropical Root and Tuber Crops (Excluding Potatoes)	252
<i>Danny Coyne and Antoine Affokpon</i>	
9 Nematode Parasites of Food Legumes	290
<i>Richard A. Sikora, Biodun Claudius-Cole and Edward J. Sikora</i>	

10 Nematode Parasites of Vegetables	346
<i>Johannes Hallmann and Beira H. Meressa</i>	
11 Nematode Parasites of Groundnut	411
<i>Patricia Timper, Don W. Dickson and Sonia Steenkamp</i>	
12 Nematode Parasites of Citrus	446
<i>Ebrahim Shokoohi and Larry W. Duncan</i>	
13 Nematode Parasites of Subtropical and Tropical Fruit Tree Crops	477
<i>J. Alfonso Cabrera and Fahiem E. El-Borai</i>	
14 Nematode Parasites of Coconut and other Palms	504
<i>Reginald Griffith, Robin M. Giblin-Davis, P.K. Koshy, V.K. Sosamma and Natsumi Kanzaki</i>	
15 Nematode Parasites of Coffee and Cocoa	536
<i>Luc Villain, Sônia M. Lima Salgado and Phap Q. Trinh</i>	
16 Nematode Parasites of Tea	584
<i>Nalini C. Gnanapragasam and Keerthi M. Mohotti</i>	
17 Nematode Parasites of Bananas and Plantains	617
<i>Richard Sikora, Danny Coyne and Patrick Quénehervé</i>	
18 Nematode Parasites of Sugarcane	658
<i>Prabashnie Vengetsamy Ramouthar and Shamsul Arafin Bhuiyan</i>	
19 Nematode Parasites of Tobacco	687
<i>Charles S. Johnson and Cleopas Chinheya</i>	
20 Nematode Parasites of Pineapple	717
<i>Brent S. Sipes and Buncha Chinnasri</i>	
21 Nematode Parasites of Cotton and other Tropical Fibre Crops	738
<i>Richard E. Davis, Rafael Galbieri and Guilherme L. Asmus</i>	
22 Nematode Parasites of Spices and Medicinal Plants	755
<i>Santhosh J. Eapen and Rakesh Pandey</i>	
23 Management Practices: An Overview of Integrated Nematode Management Technologies	795
<i>Richard A. Sikora and Philip A. Roberts</i>	
Appendix – Plant Parasitic Nematode Genera and Species Cited	839
<i>David J. Hunt</i>	
Index	853

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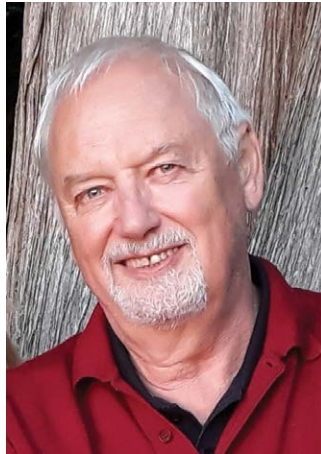
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About the Editors



Richard A. Sikora. Richard Sikora headed Nematology and Soil-Ecosystem Phytopathology at the Institut für Pflanzenkrankheiten of the University of Bonn, Germany, from 1971 until retirement in 2008. He received his BSc and MSc degrees in Zoology and Botany at the Eastern Illinois University, USA, and his PhD in plant pathology in the Department of Plant Pathology, University of Illinois, in 1970. He spent one year at G.B. Pant Agricultural University in India, where he researched the use of organic amendments for nematode management. He has worked in subtropical and tropical nematology on a global scale for a broad array of international research and development organizations. He has worked extensively with legumes, vegetable crops, cereals and banana, with emphasis on complex disease interrelationships, biological control and integrated nematode management. At the University of Bonn, he trained over 90 PhD students in nematology, with many coming from or working in the tropics and subtropics. He has published over 300 research papers, numerous book chapters and has edited and co-edited a number of scientific books and proceedings. He is a fellow of the Society of Nematologists and the European Society of Nematologists and is presently a fellow of the Stellenbosch Institute of Advanced Study in South Africa, where he leads a think tank researching the sustainable intensification of agriculture for food security in

Africa. He received the University of Ghent, Belgium, Van den Brande Award for Science; the Award of Merit of the University of Illinois and the International Service Award of the American Phytopathological Society for his contributions to international agricultural research and education. He recently received the German Scientific Society of Plant Protection and Plant Health Anton de Barry Award for his career contributions to plant protection. After retirement, he continues to work as an international consultant to a wide range of agricultural development organizations and industry globally.



Danny Coyne, with the exception of a couple of years of agrochemical field trials work in the UK, has effectively spent his working life in tropical agricultural research and development. In 1989, he worked as an agricultural extension officer, or 'Bwana Shamba', in rural Tanzania, specifically to address maize storage pests. This paved the way for his overseas career, which he has since spent traversing the African continent, working at both the national programme and international research institute levels. Graduating in Applied Biology from Liverpool Polytechnic, UK, he later achieved his MSc in Agricultural Research and Development from the University of East Anglia, UK. He was employed by the Natural Resources Institute under the Overseas Development Administration (ODA) Associate Professional Officer scheme and seconded to the Uganda National Agricultural Research Organization to assess nematode problems on root and tuber crops. With a new-found interest in nematology, he moved to the West Africa Rice Development Association (now AfricaRice) to examine the influence of nematodes on rice in West Africa. This work additionally served the basis for his PhD, which he gained from the University of Reading, UK, in 1999 while working at WARDA. After a short spell as an advisor to a Gesellschaft für Technische Zusammenarbeit (GTZ) crop pest management project in Malawi, he was offered the position of Nematologist at IITA in 2001. He continues to work with IITA, having first started in Nigeria, then Uganda and Tanzania, and currently is based in Kenya. He has an in-depth experience of working with a range of important tropical food crops such as banana and plantain, cassava, maize, rice and yam, as well as the intensive peri-urban vegetable systems. With specialization in plant nematology, his interests, however, extend to pest and disease interactions, especially within the realm of soil health, and biologically based control for nematode pests. Training underscores all of his work, whether at the farmer, technician or academic level, with numerous MSc and PhD students having passed under his furtive gaze. Students are the future of the science, however, and he takes great delight in observing their development and the expansion of the next generation of nematologists in Africa. He was recently awarded a guest Professorship at the University of Ghent.



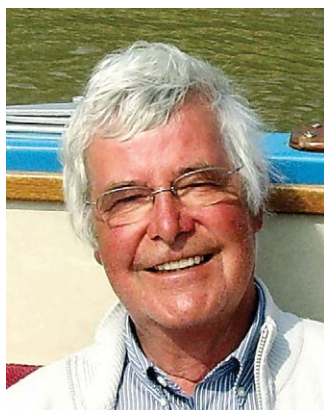
Johannes Hallmann has worked as a nematologist at the Julius Kühn Institute, Federal Research Centre of Cultivated Plants, in Münster, Germany, since 2001. His research scope includes nematode identification, taxonomy, biology, plant–nematode interactions and sustainable management strategies. Johannes Hallmann received his Diploma in Agronomy at Bonn University in 1990. He first came into contact with plant parasitic nematodes during his PhD studying the potential of endophytic fungi to control *Meloidogyne incognita* on tomato. As part of his PhD research, he monitored the occurrence of *M. incognita* and endophytic non-pathogenic *Fusarium oxysporum* on tomato in different agroecological zones of Kenya. One of the strains he isolated, *F. oxysporum* Fo162, turned out to give excellent nematode control. Supported with a stipend of the Alexander von Humboldt Foundation, Johannes Hallmann continued his research career from 1994 to 1996 as a PostDoc at Auburn University, Alabama, USA, to explore the potential of endophytic fungi controlling root knot nematodes. From there, he returned to Bonn University to work as research associate in the group of Prof Dr R.A. Sikora until he took his current position at the Julius Kühn Institute. Although his main work is on temperate nematodes, a significant part of his research is contributed to nematode problems in the subtropics and tropics, focusing mainly on root knot damage in vegetables and ornamental plants and sustainable control strategies. Research and teaching duties brought him to, among others, Myanmar, Indonesia, Morocco and Ethiopia. In 2014, he was appointed adjunct professor at Kassel University, where he teaches plant nematology in the Masters programme on Sustainable International Agriculture. Besides this, he is a guest lecturer for plant nematology at the Humboldt University in Berlin and the Rheinische Friedrich-Wilhelms University in Bonn. He supervises PhD and Master students from around the world, with many coming from subtropical and tropical countries. Johannes Hallmann received the Julius Kühn award for excellence in research honouring young scientists below the age of 40 years. He is currently President of the German Scientific Society for Plant Protection and Plant Health and Councillor of the International Federation of Nematology Societies.



Patricia Timper has been conducting research on nematodes since 1985. She obtained her BSc, MSc and PhD in Entomology from the University of California, Davis, USA, and is currently employed as a Research Plant Pathologist with the Agricultural Research Service (United States Department of Agriculture) in Tifton, Georgia. While conducting her dissertation research on fungal antagonists of entomopathogenic nematodes, she decided to switch disciplines and work with the biological control of plant parasitic nematodes. Following her PhD, she worked with fungal antagonists of the lesion nematode in potato and the soybean cyst nematode in soybean. In her current position, she applies her strong background in invertebrate ecology to solve problems critical to the sustainability of cropping systems in south-eastern USA. Her research focuses primarily on host-plant resistance, crop rotation and the biological control of root knot nematodes in groundnut and cotton. She has spent considerable effort working with plant geneticists to develop resistance to root knot nematodes in groundnut and pearl millet. Since 1998, she has worked extensively on the direct and indirect effects of cropping systems on plant parasitic nematodes and organisms that suppress nematode densities, particularly *Pasteuria penetrans*. She has been active in the Society of Nematologists (SON), serving as President (2016–2017), Program Chair (2016), Local Arrangements Chair (2012), Chair of the Nathan Cobb Foundation (2010–2013) and Nematology Newsletter Editor (2003–2005). In 2006, she received the Syngenta Crop Protection Award. She has served as Editor for several journals, including: *Journal of Nematology*, *Plant Health Progress*, *Phytobiomes* and *Australasian Plant Pathology*.



Michel Luc. Michel Luc spent his career at ORSTOM, now IRD, or Institut de Recherche pour le Développement, in the Centre of Adiopodoumé, Côte d'Ivoire, where he established the first nematology laboratory in West Africa. He conducted extensive nematological surveys in many African countries where knowledge was not available. His 18-year career in the Côte d'Ivoire ended with him being Director of the Centre. He then worked for 6 years in Dakar, Senegal, where he established another nematology laboratory. Luc developed teams of scientists and promoted research programmes in both centres. He was based in the Paris Muséum from 1975 until his retirement in 1992. He founded the *Revue de Nématologie*, now *Nematology*, and published over 150 research papers. He was Doctor *honoris causa* of the University of Neuchâtel, Switzerland, and a Fellow of the Society of Nematologists and the European Society of Nematologists. The French government made him a Chevalier de l'Ordre National du Mérite and an Officier de l'Ordre du Mérite Agricole. He also received the Médaille du mérite ivoirien.



John Bridge. John Bridge completed an MSc in Plant Pathology at McGill, Canada, and a PhD in Nematology at Imperial College, London, in 1970 and was then recruited by the UK government to be their overseas Tropical Nematologist. He was based in the UK at Imperial College and at Rothamsted Experimental Station. He lectured on the MSc Nematology course at Imperial College and supervised MSc and PhD students. In 1983, he joined CAB International as their Tropical Plant Nematology Advisor at the CABI International Institute of Parasitology. In 1998, he moved to CABI Bioscience and remained with them until retirement in 2010. During his career as Tropical Nematologist, he has helped produce four nematology books, written numerous chapters and published over 100 research papers on a wide range of nematodes on tropical crops. His work took him to a large number of countries in the subtropics and tropics on almost all continents. He has made major contributions to our knowledge of the distribution, importance and management of nematodes on tropical crops worldwide.

Foreword

The second edition of *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, published in 2005 and edited by Michel Luc, Richard Sikora and John Bridge, is now out of print. Due to a continuous demand for this book, CAB International considered an updated revision. The third edition is the result of the collaborative efforts of three highly experienced new editors – Danny Coyne, Johannes Hallmann and Patricia Timper – who added much needed experience to that of Richard Sikora. The third edition is a completely modernized book. Although largely based on the second edition, the authors have updated all aspects of the chapters by reviewing over 10,000 publications on nematodes of subtropical and tropical crops published over the past 12 years.

As in the previous edition, we have deliberately brought in many new authors – reflecting the turnover among subtropical and tropical nematologists and the need for diversity in viewpoints and experience. The multi-author chapter format was again used and authors were chosen on the basis of their practical expertise, research work and their understanding of different regions of the world, as well as their experience with a variety of crops and different types of agriculture. The number of authors has increased from 32 to 46, representing 26 different nationalities on all continents. The edition remains conceived as a truly practical book for use by agriculturists, researchers, teachers, students, extension workers and also administrators.

This new edition covers the major economically important crops of the subtropics and tropics and their main nematode parasites. As in the past, the aim was not the production of an encyclopaedia of nematode associations with crops but to concentrate on those nematode species that have been shown to cause yield loss. As in the earlier editions, the arrangement of each chapter remains practical for easy use of the information on specific topics of interest. The style of the chapters has not been modified from the original, due to the success of this structured approach in the previous editions. The text has been completely updated and revised, taking into consideration new observations, records and results published since 2005. A large number of high-quality coloured photographs have been added directly to the text, replacing the former black and white and coloured plates used in the past editions.

The book has been expanded to include a new chapter on nematode ecology, demonstrating the importance of non-parasitic nematode communities as beneficial and essential components of the agroecosystem as it relates to soil and plant health. Some chapters have been expanded upon in this edition, and this reflects the increasing importance of these crops in the tropics. Of course, some chapters have changed only slightly, due both to the loss of applied nematologists in the field and, in some cases, to the successful management practices developed in the recent past. The final chapter on management has been expanded to provide a detailed overview of efficient management strategies gleaned from the various crop chapters.

In the introductory chapter, we reflect back on the challenges and issues facing nematology in order to forecast the situation in the near future and make suggestions for change. We also have taken the prerogative to look into the future of agriculture and nematology by attempting to anticipate hypothetically the drivers of change that will influence the way nematological science is conducted in the subtropics and tropics in the years 2050 and 2100 and how nematology will be affected. Our goal is to generate discussion and to provoke thought on where we are heading as a science in the future.

It goes without saying that we are extremely grateful for the full cooperation given by the new authors of each chapter, the previous authors of these chapters and the former editors of the last two editions. Their hard work and commitment to this effort has helped to make this book a reality and we are thankful for their enthusiasm throughout the writing process.

It is important to note that CABI has decided to make the book available both in hardcover and as an e-book, creating greater access and making it even more useful for those working in applied nematology and agriculture. Conceived in this way, we hope that this new edition will again be a truly useful and practical book, not just for those dealing with plant parasitic nematodes but also for everyone working in subtropical and tropical agriculture. We wish you success in your work towards improving crop yields in a world in transformation.

The Editors
8 March 2018

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We also extend our sincere thanks to the countless nematologists, both past and present, with whom we have had the good fortune to meet and exchange views and experiences. They have been a constant source of support during the production of this third edition.

Thanks also goes to all the scientists, students and farmers who we have had the pleasure to meet, work with and to visit in numerous countries around the world. They have been an inspiration and have provided much of the information and insights into the importance of tropical nematodes and their management.

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Dedication

This new edition is dedicated to future generations of tropical nematologists.

1 Reflections and Challenges: Nematology in Subtropical and Tropical Agriculture*

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Nematology and Practical Agriculture

Agriculture is currently facing, and will continue to face, monumental challenges towards improving food production and ensuring food security in subtropical and tropical agriculture in the 21st century and beyond. The escalating rise in the human population, massive urbanization, advancing climatic change, expanding soil degradation, loss of soil fertility and depletion of water resources will have major impacts on food security, exaggerating hunger and malnutrition in many regions of the world, especially in the marginal areas of the subtropics and tropics. Nematology as a science will be confronted with even greater challenges to improve plant health and yield in the subtropics and tropics in the future than in the past. Expanding and improving nematological research in these climatic zones will be a critical part of addressing the food production and food security issues of the future.

Tropical and, to a lesser extent, the subtropical climatic zones of the world (hereafter referred to collectively as the tropics) cover large areas of the earth's land mass and are the focal point of the future problems facing humanity

(Fig. 1.1). This climatic zone is where much of the ever-increasing world population will reside and where food production needs to be improved most to nourish future generations. Productivity and yields need to be improved drastically in the tropics, where agricultural land is still available and productive and where sufficient water resources are plentiful (FAO, 2014). The tropics is where the science of nematology can have a profound impact on food crop yields in the future.

One of the major challenges facing nematologists in the tropics lies in clearly defining nematode impact and designing management solutions for family-run smallholder farms. Smallholder farms are the rule in many countries in the tropics. FAO (2014) calculates that of ~570 million farms globally, over 90% are run by an individual or a family, which relies primarily on family labour. These smallholder farms are the most common form of agriculture across the world, but especially so in the tropics. Smallholder farms occupy around 70–80% of farmland and produce more than 80% of the world's food in value terms. The vast majority of these farms are in lower-income countries, where farm sizes are already small and are becoming increasingly

*A revision of the chapter by M. Luc, J. Bridge and R.A. Sikora in the 2nd edition.

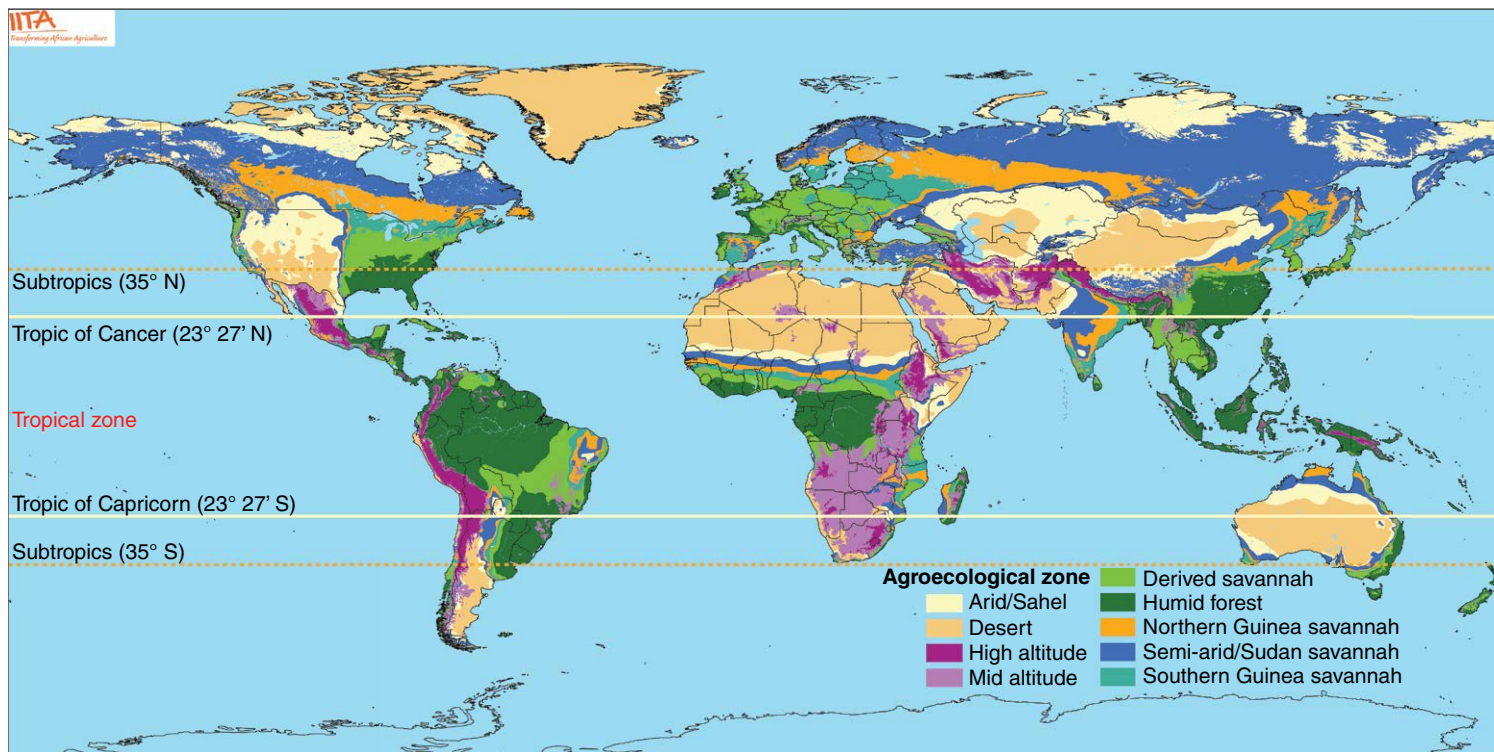


Fig. 1.1. Map of the climatic zone of the world. (From IITA, 2014. Agroecological zone map produced at Geospatial Laboratory, IITA, using zonal definition from Jagtap, 1995.)

smaller. The average farm size in China, India and Africa is 1.2 ha, and is declining in size. Worldwide, farms of less than 1–5 ha account for 94% of all farms, but control only 22% of all agricultural land. In contrast, just 1% of farms are over 50 ha, but control 65% of the world's agricultural land, mainly in high-income countries. The latter predominate in more temperate regions, but also exist in the tropics, such as for commercial sugarcane, banana or pineapple production. Solving nematology problems in the temperate zone on large-holder farms of 400 ha+, often growing a single crop per year, with modern technologies and supported by advanced research facilities, is less complex when compared to operating in the tropics, where a mosaic of smallholder farms practise year-round multiple cropping. The notion that these multiple cropping systems are simple could not be further from the fact. Factoring in the marginal/degraded state of many soils, the rapid rate of multiplication of most pests and diseases, the current low productivity, the negative impact of extreme temperature change, and the need to intensify crop production across all cropping systems reveals the sheer scale of the challenge facing both smallholder farmers and nematologists working in the tropics (see Ciancio, 2015).

Demonstrating nematode damage and solving yield decline problems under smallholder conditions has proved troublesome. Although some good examples have provided sound evidence of the huge benefits nematode management can have, such as doubling yam yields or tripling potato yields, translating this to a national or regional level proves difficult when dealing with such complex cropping systems, especially in the face of the less than comprehensive nematode data (Nicol *et al.*, 2011). Consequently, if we attempt to anticipate the future impact of nematology on production in the tropics (see Table 1.3), we may conclude that to solve nematode problems adequately, even though suitable technologies exist for effective management, may not be possible. However, if we can expect that these farms will expand in size as food security and hunger issues become more severe, it is conceivable that effective management systems can be designed.

Due to a range of constraints, research in nematode management in the tropics often focuses on low-input methods involving crop

rotations, multicropping, adjustment of planting and harvest dates, use of various soil amendments and mulches, trap and antagonistic crops, fallow and flooding, as outlined in Chapter 23, this volume. While emphasis on these forms of management strategies in the tropics reflects increased awareness of nematode damage, it also demonstrates limited availability and/or knowledge of resistant cultivars, and effective use of nematicides. However, management tools have been developed that broaden the integrated pest management toolbox, including grafting resistant rootstocks, biological control, remote sensing and precision farming, as well as new formulations and application technologies for nematicide seed treatment. Cultivars resistant to various nematodes are becoming more available, and nematicides with new mechanisms of action are entering the market. But again, access to these technologies by smallholder farmers in tropical regions is limited, because knowledge on the performance of such technologies is often not available until long after their introduction elsewhere, and financial resources for purchase are lacking. The challenge is great, but not unsurmountable.

Nematodes play a major role in causing yield loss in many crops, as outlined in the chapters in this volume. This book deals with plant parasitic nematodes attacking tropical crops and outlines the management practices that need to be implemented in the tropics to solve the food security issues of the future, regardless of farm size.

Historical Background

The first attempts to bring together information concerning the impact of plant parasitic nematodes on tropical crops were made with the compilation of the books by Smart and Perry (1968) and Peachey (1969). Until then, nematological literature focused primarily on temperate crops. The knowledge generated in the tropics was limited to the research findings of individual scientists who, for the most part, worked in isolation, often on a single crop. Charappa (in Peachey, 1969) states that our knowledge of the importance of nematodes in the tropics lags behind that available in temperate climates because nematologists and appropriate research laboratories are insufficient or non-existent in the

humid tropics where, paradoxically, plant parasitic nematodes and their damage are greatest. The importance of nematodes on subtropical crops fared far better, due to the extensive research conducted in large-holder commercial agricultural regions of Australia, Brazil and the USA, as well as in the countries bordering the tropics in southern Europe. The first review article on the plant nematology problems of tropical sub-Saharan Africa was published by Taylor (1976). He discussed the major nematodes causing crop damage on the continent. It was among the first attempts to highlight the significance of the problems facing the few nematologists working in Africa at that time.

Approximately 76,000 scientific publications on plant parasitic nematodes have been printed between 1915 and 2017 (Anon., 2017). The number, especially of tropical species, has increased steadily. During this span of time, 26,000 publications on tropical species of *Meloidogyne* have been published (14,000 on *Meloidogyne incognita* and 5000 on *Meloidogyne javanica*), as compared to the temperate root knot nematodes (2493 on *Meloidogyne hapla* and 348 on *Meloidogyne chitwoodi*). The vast majority of publications on temperate nematodes focused on species of *Heterodera* (13,000) and *Globodera* (5000). Endomigratory species of *Pratylenchus* are mentioned 8000 times and *Radopholus* and *Rotylenchulus* 2177 and 2544 times, respectively. The number of publications on a 10-year basis since 1930 dealing with all plant parasitic nematodes, and with root knot specifically, has increased steadily (Fig. 1.2).

The first edition of the book by Luc *et al.* (1990) provided the first major attempt to review and document the considerable knowledge accumulated on the presence, symptomology, importance and management of plant parasitic nematodes on major food crops in the tropics. In addition, chapters on plant parasitic nematodes affecting tropical crops have been provided in numerous reference and text books, and are cited repeatedly in this revision.

Tropical–Temperate Interaction

We often refer to nematology in temperate compared with tropical climatic zones. It is appropriate here to raise the obvious questions of whether

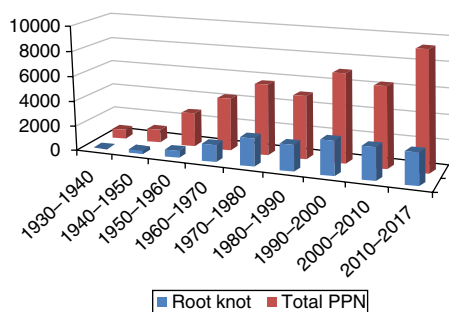


Fig. 1.2. Number of published papers dealing with plant parasitic nematodes in general, and with species of *Meloidogyne* as listed in CABDirect between 1930 and 2017.

Note: PPN = plant parasitic nematodes.

there are fundamental differences or whether they differ only because of the different species of nematodes and types of crop present. Some of the fundamental differences have been discussed in detail by Noe and Sikora (1990) in the first edition of this book. It should be noted, however, that conditions akin to temperate climates prevail in some of the higher-altitude areas, as well as during the cooler rainy seasons in the tropics. Therefore, while there is geographical latitudinal division of the climatic regions, areas within these latitudes differ according to altitude (see Fig. 1.1).

Plant health management is a never-ending continuum of challenges, particularly as it relates to nematode detection and management. These challenges to nematology in the tropics are multidimensional, involving interacting biological and abiotic factors. The fact that not all things are created equal means that the accumulation of knowledge on nematode damage and the improvement of management practices does not develop at the same speed worldwide between these two climatic zones. Agricultural production and nematode management in the tropics has consistently lagged behind the level of expertise attained in the temperate regions.

In the previous edition of this book, we stated with some certainty that most, if not all, major problems caused by nematodes in temperate countries have been detected. We believe that this fact stands today, and that new nematode problems of major significance have not been detected since 2005. Forgotten

problems have reappeared as rotation sequences are altered or new cultivars introduced, as seen with new outbreaks of the potato cyst nematode, *Globodera pallida*, and the sugarbeet stem nematode, *Ditylenchus dipsaci*. In addition, molecular diagnosis has identified new species that need study.

A problem new to a particular tropical country could arise through the introduction and subsequent spread of a known nematode parasite from another country, such as for *M. hapla* on cut flowers in Ethiopia or *Globodera rostochiensis* on potato in Kenya, both presumed to have arrived from Europe with infected planting material (Meressa *et al.*, 2014; Mwangi *et al.*, 2015). Introductions will no doubt continue to occur, especially as quarantine services, porous borders and nematology expertise are often ill equipped to prevent new introductions.

Although a major challenge for nematologists working in the tropical versus the temperate zone pertains to the complexity of smallholder farms, having limited access to finance, production inputs and extension services etc., the warmer year-round climate in the tropics definitely influences nematode distribution and epidemiology, since most nematode life processes have thermic optima that determine the ideal geographic ranges of nematodes. Presumably, there are southern and northern hemisphere bands of appropriate temperatures for each nematode species that would be contiguous and would meet at the equator for true tropical species. This is clearly shown for the distribution limits of *M. javanica* versus *M. incognita* in southern USA, a pattern that does not seem to be reflected in Africa (Sasser and Carter, 1985). We know very little about the distribution patterns of many economically important nematodes of tropical crops, especially in countries where small farms predominate. Temperature with increased altitude, however, does influence nematode populations on banana grown in eastern Africa (see Chapter 17, this volume).

The negative impact plant parasitic nematodes have on agricultural production in the tropics is immense, and the overall amount of damage and consequent impact that nematodes have on yield is repeatedly underestimated. This miscalculation is likely to increase as the science of nematology in the tropics trails behind that of temperate regions, as well as behind other

scientific disciplines as the world struggles to negate the increasing food deficit.

Emerging Threats

New problems continue to be discovered in the tropics, involving new nematode species, even genera, or species not previously recorded as harmful to a crop. Examples include 'legume Voltaic chlorosis' on leguminous crops in Burkina Faso, caused by a new species, *Aphasmatylenchus straturatus*, from a genus not previously known to be harmful (Germani and Luc, 1982); 'miti miti' disease of taro (*Colocasia esculenta*) in the Pacific caused by a new species, *Hirschmanniella miticausa* (Bridge *et al.*, 1983); in the semi-arid areas, the new cyst species *Heterodera ciceri* causing damage to chickpeas and lentils (Greco *et al.*, 1984; Vovlas *et al.*, 1985); *Ditylenchus africanus* on groundnut (Jones and De Waele, 1988); *Achlysiella williamsi*, a new genus and potentially damaging pest of sugarcane (Hunt *et al.*, 1989); *Meloidogyne paranaensis* (Carneiro *et al.*, 1996), now a devastating pest on coffee in Brazil; *Radopholus duriophilus*, associated with the decline and death of durian trees and widely distributed across Vietnam (Nguyen *et al.*, 2003); *Radopholus arabocoffeae*, described from Arabica coffee, and *Radopholus daklakensis* from Robusta coffee in Vietnam (Trinh *et al.*, 2004, 2012). *Meloidogyne izarcoensis* was described from coffee in El Salvador (Carneiro *et al.*, 2005), where it apparently has a relatively small, limited area of distribution on coffee (Villain *et al.*, 2013), but then is discovered on coffee in East Africa and on tomato and cabbage in West Africa (Jorge Junior *et al.*, 2016). Recently, a new genus of sedentary Pratylenchidae was described, *Apratylenchus*, with two species, *Apratylenchus vietnamensis* and *Apratylenchus binhii*, found on coffee in Vietnam (Trinh *et al.*, 2009). New species of *Meloidogyne* continue to be described but *Meloidogyne enterolobii* (= *Meloidogyne mayaguensis*), a highly aggressive and potent nematode (Castagnone-Sereno, 2012), requires particular note. First described only in 1988 (Rammah and Hirschmann, 1988), it has since been recovered consistently from various crop hosts, from various locations across the tropics on a regular basis, such as on guava in Brazil, Thailand and Vietnam, yam in Nigeria, sweet potato in Kenya,

cowpea in Mozambique, banana in China, etc. (see relevant chapters this volume), and poses a severe threat to the production of numerous crops. As we start to look, we begin to find! Many of these new threats are the direct result of developing and supporting nematology expertise in countries where it did not exist or was not strong previously. De Waele and Elsen (2007) discussed the potential of a number of already identified species with future importance should they expand their distribution through international trade.

We can expect new, and as yet unseen, nematode problems to emerge as agricultural production changes; for example, the steady shift in rice production from irrigated to upland will result in the emergence of new nematode problems. Similarly, the promotion of soybean production into Africa will undoubtedly lead to damage by known and unknown nematode species. Determining the solutions to these types of problems could be hampered by inadequate access to suitable facilities and competence in systematics.

There is little doubt that many more undescribed nematodes and their associated problems are yet to be discovered across the tropics, as nematology expertise improves, activities are initiated or expanded and as new crops are introduced into new areas. Whether or not these findings will simply lengthen the list of known species, or whether it will contribute to our knowledge of nematode biodiversity and systematics, and help growers with significant damage problems, is open to question and dependent on the reach of nematology into the tropics. Other nematodes that are still limited in distribution but could become important and needing scrutiny by quarantine agencies are listed in Chapter 23, this volume.

Global Spread and Quarantine

The ever-increasing movement of agricultural produce, either in dried or fresh form, as well as seeds and planting material, ensures the continued and future dissemination of nematode pests, underscoring the need for trained nematologists within phytosanitary and quarantine institutions. The limited number of nematologists in quarantine positions can have a negative

impact on the development of quarantine guidelines, besides affecting the prevention of nematode introductions into previously non-infested areas. Good examples are the dissemination of the banana burrowing and root lesion nematodes (*Radopholus similis*, *Pratylenchus* spp.) and of the citrus slow decline nematode (*Tylenchulus semipenetrans*) to nearly all areas where these crops are grown, as well as the movement of *G. rostochiensis* into the high-altitude tropical growing areas of the Philippines (Sikora, 1982) and Kenya (Mwangi *et al.*, 2015). The spread of known, economically important plant parasitic nematodes has occurred in the recent past, and will continue as long as we cultivate crops and move agricultural produce around the globe; for example, the spread of *Heterodera glycines* to South America, *G. pallida* to Europe and *Bursaphelenchus cocophilus* to Central and South America.

The detection, description and recognition of possible new species of nematodes, often in multispecies mixtures, is highly relevant to practical nematology and quarantine departments. For example, a large number of nematodes are of potential importance to quarantine globally (Chapter 23, this volume). In many cases, these less-known species will go undetected through a lack of trained nematologists, thus resulting in their spread. Good examples are: *Pratylenchus coffeae*, *Pratylenchus goodeyi*, *M. chitwoodi*, *Meloidogyne graminicola*, *M. enterolobii*, *Meloidogyne floridensis*, *D. dipsaci* and *B. cocophilus*, to mention but a few of the better-known species. In addition, a system for detecting races and virulent populations that are able to break resistance in cultivars will be required, complicating phytosanitary efforts further. Improved communications and web-based information portals will help extend such information, but will not overcome the deficit of trained nematologists to physically deal with problems.

Diagnostics

The use of DNA fingerprinting for nematode diagnostics has been discussed extensively by De Waele and Elsen (2007). The vast majority of work was undertaken with nematodes important to quarantine in the temperate regions of the world, i.e. species of *Heterodera* and *Pratylenchus*,

as well as the most important tropical species of *Meloidogyne* and *Radopholus*. There is a dire need to expand these diagnostic tools to tropical nematodes affecting other crops. Quick and accurate identification to species level is also important for the planning of management programmes based on resistance. De Waele and Elsen (2007) emphasize that the improved use and knowledge of molecular diagnostics by nematologists in the tropics lags behind, due, in part, to the lack of available equipment and resources to support such work. The situation is changing, however, as advanced laboratories with significant financial and technical support become established, such as EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) in Brazil, ARC (Agricultural Research Council) in South Africa, BecA (Biosciences eastern and central Africa) in East Africa, as well as with CGIAR (Consultative Group for International Agricultural Research) and in centres within progressive and advanced national programmes. De Waele and Elsen (2007) estimate that perhaps just 10% of nematode biodiversity has been elucidated, and consequently the characterization of many more new nematode species is to be expected, especially from the tropics. They state that without increased research opportunities and international collaboration, tropical nematologists will have few prospects for participating in the application of molecular diagnostics to nematode surveys or the analysis of the origin and dispersal of nematode species based on molecular diagnostics. In addition, uncertainties concerning the validity of nematode species, unless resolved, will present practical problems related to quarantine measures.

There is a greater diversity of nematode genera and species in the tropics compared with temperate climates. As many of these nematodes are new taxa, it is evident that there is a great deal of work for nematode taxonomists in the tropics. This, indeed, is happening, but a big disadvantage of concentrating on the taxonomic aspect is that the surveys are designed to collect nematodes and not to determine the problems they cause. This is often the only possible means of establishing new nematology laboratories with limited staff and financial means. The danger is that such laboratories can limit their activities to systematics and so become production lines for new species and genera, to the exclusion

of determining the importance of the nematode being described. The reality is that each supports the other, and one without the other is severely constrained in their ability to perform.

Establishing Pathogenicity

The identity of nematodes parasitizing crops is an obvious first and necessary step, but establishing their importance as pests in tropical agriculture needs to be a key priority. A big obstacle to achieving this, however, is that in the tropics nematodes more often occur as mixed communities, creating difficulties in elucidating the pathogenicity of the individual species present. The practical problems of determining nematode pathogenicity in the tropics can often be far more difficult than in temperate countries (Noe and Sikora, 1990).

Of course, many nematodes are now recognized as serious or potentially serious pests of tropical crops, as detailed in the following chapters. In our opinion, while the vast majority of plant parasitic nematodes that are economically important in temperate zones have been studied in detail and their pathogenicity well established, this is far from complete in the tropics. The presence of multiple species complicates differentiating individual species pathogenicity to begin with, while solid data using controlled studies to determine damage and yield impact on various crops for various nematodes remain wanting. In some cases, the lack of information on some crops is quite astonishing. Take coffee, for instance: in Brazil, the nematode problem is viewed as a serious constraint and has nematologists dedicated to working solely on the crop, with state laws in place even to ensure nematode-free seedling certification. In Africa, where coffee is a key commercial commodity in a number of countries, there is scarce information on species distribution and occurrence and only limited nematological activity (see Chapter 15, this volume). Yield loss estimates, caused by plant parasitic nematodes across a wide range of crops, were listed by Sasser and Freckman (1987) based on information provided by 371 nematologists from 75 countries (Table 1.1) and provides a guide to potential yield loss for nematodes even today. The levels of damage on susceptible crop cultivars as shown in that table has arguably

Table 1.1. Summary of estimated yield losses due to damage by plant parasitic nematodes worldwide. (From Sasser and Freckman, 1987.)

Crop	Loss (%)	Crop	Loss (%)
Aubergine	16.9	Oat	4.2
Banana	19.7	Okra	20.4
Barley	6.3	Papaya	15.1
Cacao	10.5	Pepper	12.2
Cassava	8.4	Pigeon pea	13.2
Chickpea	13.7	Pineapple	14.9
Citrus	14.2	Potato	12.2
Coconut	17.1	Rice	10.0
Coffee	15.0	Sorghum	6.9
Cotton	10.7	Soybean	10.6
Cowpea	15.1	Sugarbeet	10.9
Field bean	10.9	Sugarcane	15.3
Grape	12.5	Sweet potato	10.2
Groundnut	12.0	Tea	8.2
Guava	10.8	Tobacco	14.7
Maize	10.2	Tomato	20.6
Melon	13.8	Wheat	7.0
Millet	11.8	Yam	17.7

changed little, and this level of damage is to be expected wherever plant parasitic nematodes attack these crops (Nicol *et al.*, 2011). Crop losses caused by nematodes have also been estimated by nematologists in the USA for temperate and subtropical crops (Koenning *et al.*, 1999). The chapters in this book contain pertinent and up-to-date information on yield loss caused by important nematodes of the most widely cultivated crops in the tropics. Each chapter documents the extent of damage that is, or could be, caused by nematodes as recognized by nematologists. This documentation is important, to enable informed and balanced decisions for crop protection and management options. Unfortunately, nematodes are a largely overlooked and neglected aspect within the agricultural and crop protection sector. Crop damage by nematodes invariably remains hidden, due to the many other limiting factors operating in tropical agriculture, especially the presence of multiple biotic and abiotic stress factors operating simultaneously on the crop. Nematodes have rarely been considered or recognized as major limiting factors until all other constraints on yield increase have been removed (Bridge, 1978). Much effort, therefore, remains for nematologists to increase awareness, knowledge and understanding of nematode problems and towards developing appropriate

approaches to sustainable management, especially for smallholder farmers. Greater dissemination of information to farmers, agricultural scientists, extension officers and administrators is necessary to implementing practical and economic means of controlling nematodes, in the face of all the other constraints on crop production.

Yield Interactions

Understanding and unravelling the interactions between nematodes and biotic as well as abiotic factors warrants much greater emphasis and needs to be placed at the forefront of the research agenda. Nematodes are never present in isolation, but act in combination with both biotic and abiotic factors, all of which have a compounding effect on crop damage. For example, nematode-infected plants are often severely damaged when crops are cultivated under conditions of drought and/or nutrient stress in the tropics, and especially under tropical dryland semi-arid conditions. In many areas of the world, where legumes are grown on degraded soils with marginal inputs, nematodes significantly intensify yield depression. Nematodes induce vascular disorders and reduce root penetration of the soil profile by infecting and inhibiting the primary root development of seedlings. The resulting nematode-induced shallow root architecture increases the negative impact of moisture stress on root health and yield (see Chapter 23, this volume). This is a reality for many smallholder growers and goes unnoticed by most agriculturalists. Banana, plantain and root and tuber crop production is heavily affected by these types of interactions (see Chapters 8 and 17, this volume). Nematodes and fungi acting in concert are responsible for severe root rotting of both primary and secondary roots, causing toppling of banana and yield loss in many crops. Similar interactions exist between nematodes and root pathogens adversely impacting root architecture of wheat and maize (see Chapter 6, this volume).

Climate Interactions

There are more intrinsic differences between temperate and tropical areas, based mainly on

the wide diversity of nematodes, crops and agricultural systems. The range and severity of parasitism on all living organisms, humans, animals and plants, is greater in the tropics. Plant parasitic nematodes generally have shorter life cycles, resulting in a more rapid population increase than in temperate areas. For example, in temperate areas, *Heterodera* spp. generally produce one or two generations per year, whereas *Heterodera oryzae*, in West Africa, produces one generation every 25 days (Merny, 1966). Conversely, the 4- to 8-week duration of a life cycle of the northern root knot nematode *M. hapla* compared with the tropical species *M. incognita* and *M. javanica* are similar to each other, whereas *M. graminicola* on rice completes a life cycle in less than 20 days. Since the completion of a life cycle is usually temperature dependent, as global climates become warmer, the number of life cycles will increase on a per season basis in the tropics, adding to the importance of nematodes as limiting factors. The damage caused will reduce root penetration in soil, cause deformation in root architecture and thereby decrease yields. In the distant future, nematologists will be confronted increasingly with these types of biotic–abiotic interrelationships and their effects on root growth, and thereby yield.

Multispecies Infections

Crops are often attacked simultaneously by a number of economically important nematodes. In temperate areas, there may be ‘secondary species’, but most often there is one main, easily recognizable nematode parasite of a crop, on which control efforts can be focused. This is not the case for many tropical crops, where a number of species of several different genera may be major parasites of a crop.

Sugarcane is a good example, which can be affected by 10–20 different species in a single field, such as *Meloidogyne*, *Heterodera*, *Achlysiella*, *Pratylenchus*, *Xiphinema* and *Paratrichodorus*. Multispecies nematode infections have a number of consequences concerning their control. First, they can seriously hinder the establishment of an effective crop rotation, as the host status of each crop will differ depending on the nematode species present. A good example of this phenomenon was experienced in Côte d’Ivoire,

where *Crotalaria* was recommended as an intercrop to control *Meloidogyne* spp. on pineapple. The intercrop provided effective control of the root knot nematodes, but increased *Pratylenchus brachyurus* densities to levels that were at least as harmful to the crop as *Meloidogyne* spp. Other such examples include the nematodes parasitizing wheat, where multiple species of *Heterodera* are often found with multiple species of *Pratylenchus* (see Chapter 6, this volume).

Multispecies infections further complicate the search for host resistance against nematodes; targeting one nematode species, while proving potentially useful, is normally not sufficient in the tropics. This is very clear in wheat, where cyst nematodes and root lesion nematodes are found concomitantly along with root rotting fungi (see Chapter 6, this volume). Similarly, in both commercial and smallholder banana production, a range of foliar and root diseases combine to exaggerate losses in fields infested with multiple species of important nematodes (see Chapter 17, this volume). The lesson of breeding for resistance to one species of nematode should have been learned following the emergence of the potato cyst nematode *G. pallida* following the development of *G. rostochiensis* resistant cultivars. The recent detection of a new and aggressive species of root knot, *M. floridensis*, which was detected because it was not parasitized by the obligate bacterial parasite, *Pasteuria penetrans*, should also be mentioned. Strong differences in the level of aggressiveness among populations of *R. similis* attacking banana will also affect future integrated pest management strategies.

Management

The most fundamental differences between tropical agriculture and temperate agriculture, which markedly affects the study and control of plant parasitic nematodes, are the crops that are grown, the cultural practices and the farming systems. A substantial proportion of crops in the tropics are propagated vegetatively, in contrast to the dependence on seed-reproduced plants in temperate zones. This makes a profound difference in terms of the dissemination of nematodes, through untreated infected planting material. Commercial plantation crops are a common feature of tropical agriculture, but by far the largest proportion of

cultivated land is farmed on smallholdings, using an exceptional range of crops, including cash and utility crops, through traditional cropping practices with limited external inputs. The outstanding feature of traditional agriculture is the complexity of cropping systems and the cultivation methods involved (Bridge, 1996). In contrast, modern farming in temperate zones is comparatively uncomplicated, and the study and control of the nematodes is also, in comparison, relatively straightforward. The many different farming systems operating in the tropics fall into four main categories: (i) shifting cultivation; (ii) lay or fallow farming; (iii) permanent cultivation; and (iv) multiple cropping. In some of these farming systems, nematodes are less likely to be causing damage; in others, the cultivation practices will enhance greatly the risk of nematodes causing serious yield losses (Bridge, 1987).

The nematode management methods that theoretically can be employed in tropical countries differ little from those used in temperate zones, but in practice they are more difficult to implement and need to be modified considerably in many circumstances (see Chapter 23 and individual chapters, this volume). There will be obvious differences in nematode management between low- and high-income countries and between large-scale commercial farms/plantations compared with smallholder farms using traditional cultivation systems.

Chemical nematicide treatment of soil has been a recognized means of nematode control in temperate zones and for large-scale commercial crop production in the tropics. The removal from use of many of these products has, however, shifted focus to alternative means and options, and in many ways has driven the search for more suitable alternatives. However, for most smallholder production systems in the tropics, synthetic chemical pesticides are not used, nor relied on, except for some cash crop and perishable vegetable production. Limited awareness of pest and disease causal agents, which products to apply, and a less than efficient pesticide supply chain lead to the misuse and abuse of pesticides. Correcting this serious problem requires a consolidated effort on the part of nematologists. Studies on vegetable production systems, targeting the use of healthy seedlings to remove root knot nematode infection and other diseases, compared with smallholder traditional farmer

seedling systems significantly reduced overall pest and disease levels and the need for pesticides, resulting in higher, better-quality yields (see Chapter 10, this volume). Knowledge of nematode problems and addressing them can consequently have far-reaching effects, with multiple benefits. Nematicides with new modes of action that are effective and environmentally acceptable are now entering the market. The development of new and targeted forms of application technology, for example seed coating or application through drip-irrigation systems, is now well developed. Many of the compounds on the market have systemic activity. It should be noted that when applied at the recommended doses, many, if not all, of these compounds will protect the plant in the pathozone by the inactivation or mortality of nematodes, but will not reduce nematode population densities in the field unless repeated applications are performed.

The modification of existing agricultural practices in order to manage nematodes is one of the most acceptable alternatives to chemical control for both smallholder and large-scale farmers in the tropics. Crop rotation can vary from non-existent, where there is continuous cultivation of susceptible, sequentially planted crops, through what can be termed random rotation, to a relatively sophisticated form of rotation. However, most rotation schemes in operation have been designed to prevent disease outbreaks, reduce risk or increase available nutrients, and are not always compatible with nematode control. With an understanding of the nematodes involved and the accepted cropping systems, modifications can be made to produce effective control by the rotation of crops. In order to develop or recommend rotations, including the use of resistant cultivars, accurate information on the nematode species present is necessary. The absence of such information undermines our ability to make recommendations confidently. Many cultural methods, apart from rotation, can be used, and are outlined in the following chapters.

Resistant Cultivars and Genetic Modification

Host resistance produces the most dramatic increases in the yield of many crops and, when available, provides a major and desirable solution

to nematode problems. This is particularly true with regards to the problems facing smallholder farms where other inputs are inaccessible. Wherever resistant cultivars are available, their use should be recommended, but, importantly, they should be used in concert with other integrated control techniques to avoid the selection of resistant-breaking populations, a phenomenon encountered with *G. pallida*, *H. glycines* and *M. incognita*. However, in many, if not most, of the crops covered in this book, there is little or no known source of resistance for breeding programmes. For vegetatively propagated crops, breeding for new traits can also be very protracted and frustrating. Banana and yam, for instance, both pose major challenges for breeding, given their polyploidy, low fertility, seed set and germination, combined with limited sources of resistance.

A number of difficulties affect the introduction of resistant cultivars to tropical countries. A major hurdle is the practical introduction of these cultivars, with bureaucratic release policies and often lengthy and physical supply networks that are cumbersome or disorganized in some countries. Where resistant cultivars are available and suited to the conditions prevailing in a country, many other factors need to be taken into account. For instance, smallholder farmers, and often agricultural staff, are unaware that nematodes comprise numerous species and are not just a single species. Often, these farmers are completely unaware of nematodes in the first place. Consequently, a label stating 'resistant to nematodes' does not provide them with the blanket control they are expecting. And critical to this is knowledge on the nematode species (or strain) of concern. A major vegetable export company in Ethiopia, for instance, grafted a costly, high-yielding hybrid cultivar on to 'nematode-resistant' rootstock imported from Europe to control a root knot nematode problem. This failed due to incorrect diagnosis of the *Meloidogyne* species present versus the resistance conferred by the rootstock, at great cost to the farm. Farmers are generally unaware that resistance to one species of nematode does not necessarily mean resistance to others, or that the *Mi* gene in tomato breaks down at high temperatures. One of the successes made to date for managing nematodes worldwide is resistance to genera belonging to the Heteroderidae, which have a

highly developed host–parasite relationship where cell modification occurs and is required for successful reproduction of the nematodes (Luc and Reversat, 1985). However, many tropical plant parasitic nematodes belong to migratory endoparasitic groups, which cause cell destruction without modifying the host tissues and may be more difficult to control with gene modification; for example, species in the genera *Radopholus*, *Pratylenchus*, *Hirschmanniella*, *Scutellonema* and *Hoplolaimus*. In the tropics, the occurrence of multiple nematode species on individual crops can also render single species resistance of limited use, as one damaging species can come to dominate relatively quickly with the suppression of another. There will again be a marked contrast in what can be achieved with the commercial producer compared with the smallholder farmer, but consideration has to be given to both. A good illustration of this difficulty followed the introduction of dwarf rice cultivars to prevent lodging in South-east Asia (Mydral, 1974), which did not account for local smallholder needs, depriving people of their usual source of rice straw for animal feed, bedding and thatching.

The recent development of transgenic plants with resistance to insects, and the detection of genes in the plant that are responsible for giant cell formation, as well as genes in plants needed for protein synthesis by the nematodes, may lead to new forms of resistance. The importance of this technology to smallholder and commercial growers, to the different nematode groups and crops, although highly publicized, will take years to have an impact, as well as trickling down to the smallholder growers. The cost and duration of developing transgenic crops will undoubtedly outlive the current edition of this book.

There was, and remains, a degree of optimism concerning the future use of genetic engineering for nematode resistance. This approach would be especially important where sources of resistance are not known in the target crop. Another key advantage is that culturally preferred cultivars can be targeted, ensuring ultimate market acceptance in respect to preferred traits. Gene manipulated lines with resistance to nematodes have been developed (Lilley *et al.*, 2011; Roderick *et al.*, 2012; Ali *et al.*, 2017), with some promising reports of nematode control (Atkinson *et al.*, 2012; Tripathi *et al.*, 2015). Unfortunately, this technology has yet to result in marketable

cultivars, due in part to the lengthy process of developing biosafety regulations for each country concerned and consumer acceptance of the products of this new technology. At the present time, there seems to be a lull in reports of progress.

Research Centres and Capacity Building

The first nematology laboratory established in the tropics was in West Africa (by ORSTOM (Office de la Recherche Scientifique et Technique d'Outre-Mer) in Côte d'Ivoire) in 1955, when just nine published references relating to plant parasitic nematodes were available for the whole of West Africa and Zaire. With the strong support of the nematologists working in the UK, a thrust was made to develop the field of nematology in many of the Commonwealth countries during the 20th century. The first laboratories were established in India and Kenya, with a great deal of our initial information on nematodes from the tropics gained from these countries.

Nematology centres have since been established in a number of tropical countries around the world. In addition, a number of research hubs exist today, where teams of nematologists are linking closely with institutions and advancing nematology collaboration. Such hubs are now found in Brazil, Egypt, India, Israel, Italy, Kenya, Mexico, Nigeria, Pakistan, South Africa, south-east, south and south-western USA and Spain. Within the International Agricultural Research Centres network (CGIAR), IITA (International Institute of Tropical Agriculture) and CIMMYT (International Maize and Wheat Improvement Center) nematologists are important drivers in coordinating and stimulating research on food crops in Africa and Asia. A significant amount of collaboration exists between scientists working in subtropical and temperate countries who are conducting research and supporting capacity building in the tropics.

Nematology laboratories established in the latter half of the 20th century in tropical regions needed to take a fresh look at nematode problems. Basic survey work was often (and still is) necessary to determine initially which problems existed and to identify accurately which nematodes were present (determination, systematics), followed by establishing which nematodes were harmful or economically important, and finally

deciding on which treatments or methods were appropriate for their management. Many major problems involving nematodes now have recognized management options available, while research efforts are required for others, in particular where multiple species occur in the same crop or field. Some examples include the *Pratylenchus* spp.–root rotting fungi complex on East African Highland bananas and plantains; multiple species infections of lesion and ectoparasitic nematodes on African and Asian tuber crops; the development of virulent populations of potato and soybean cyst nematodes; as well as severe problems with the cyst–root lesion nematode complex on wheat in North Africa, the Middle East and West Asia. The latter complex is extremely damaging, due to interactions with root rotting fungi (see relevant chapters, this volume).

There are an estimated 1600 active nematologists registered as members of the major societies of nematology in India, Brazil, Pakistan, Nigeria, the USA (SON, Society of Nematologists), EU (ESN, European Society of Nematologists) and Latin America (ONTA, Organization of Nematologists of Tropical America) (R.A. Sikora, University of Bonn, Germany, 2017, personal communication). The present trend of downsizing in all fields of agricultural research needs to be rectified. The disappearance of diagnostic laboratories and qualified taxonomists is a problem that is especially important as it relates to quarantine, where decisions on nematodes detected in samples, in particular species' and virulent populations' designations, need to be made almost spontaneously.

The number of MSc and PhD students from the tropics studying nematology in major universities worldwide has increased drastically, and these students are now offering nematology programmes in national universities worldwide. A major thrust to promote capacity building was through the establishment of the PINC MSc programme (Postgraduate International Nematology Course) at the University of Ghent, Belgium. The programme has trained over 300 nematologists since its inception in 1992, the vast majority coming from and returning to the subtropics and tropics. The programme is presently expanding to include satellite modules based in Ethiopia and Kenya to better serve the demands of subtropical and tropical nematology.

Between 2005 and 2010, an advanced research and training programme was initiated to

build capacity in plant nematology in East and Southern Africa funded by the Gatsby Charitable Trust. Research centres in Kenya, Uganda, Tanzania, Malawi and Zimbabwe collaborated with Rothamsted International, the University of Reading and CABI Bioscience in the UK to improve nematological research in eastern Africa. This programme was important in that it not only developed capacity but also contributed significantly to the upgrade of laboratories, with much needed equipment. Advanced research and training agricultural programmes with a strong emphasis on nematology were also developed in Brazil (Souza, 2017) and South Africa (Fourie *et al.*, 2017), with both programmes supported by very active nematology societies.

Stronger links need to be established between nematologists working in the tropics, both scientifically as well as financially. The possible formation of a new international consortium with multiple country funding needs to be promoted. In the future, it may be necessary to develop ‘virtual hubs’ or ‘centres of excellence’ in diagnostics for use by nematologists, breeders, agronomists and extensionists working in the tropics to support nematology in the field of species identification. The use of modern computer and digital imaging may allow access to existing centres of competence in systematics, for example in Ghent (Belgium), Aligarh (India), multiple laboratories in the USA, Braunschweig (Germany), Bari (Italy), Wageningen (the Netherlands), Sophia Antipolis (France) and EMBRAPA stations in Brazil, to name a few. There is scope for building on the fast-developing use of digital imaging for virtual diagnosis via electronic means.

Funding

Nematology research in the tropics is underfunded, and there remains a shortage of nematologists to work on problems. Sasser and Freckman (1987) estimated that less than 0.2% of the crop value lost to nematodes worldwide was invested to fund nematological research to combat these losses, a value that likely exceeded US\$100 billion annually. In our opinion, support has, if anything, decreased over time, due in part to the overall reduction in emphasis on funding for agricultural research worldwide, but

notwithstanding a lack of visibility of the science with donors and its consequent (inevitable) neglect.

Furthermore, the percentage of funding for nematological research in the tropics is considerably less than it is in most temperate countries, making the amount infinitesimal. Examination of the senior scientific staff in the CGIAR Centres over a 20-year period, for example, showed that while overall numbers of scientists increased markedly, nematologists remained unchanged at a bare minimum, and proportionately becoming much less (Sharma *et al.*, 1997). Since 1997, the situation has changed little, and within the CGIAR today there are but a handful to cover the seemingly escalating nematode problems. This is important, in that these nematologists act as a crucial link within the scientific community in areas where working conditions can be very challenging (Coyné *et al.*, 2014).

The need for research in tropical agriculture is greater than ever before, with the extreme problems facing food production in smallholder farms, in addition to the considerable losses in commercial farms. Many temperate countries are suffering the embarrassment of massive food surpluses, which are not transferable or only at a high cost to the less developed world. The majority of countries in the tropics have shortfalls in the production of most crops that can exceed 80% of that attained in temperate agriculture. Improved crop productivity and food security is necessary to raise the nutritional level of burgeoning populations, as well as to the economy through important export crop commodities. For the most part, this increase in production can be reached even at the small farm level, using the often lacking good farming principles. Solving nematode problems, on the other hand, will play an important part in improving crop yields to the benefit of all farmers, and thereby consumers and the national economy. It falls to each and every nematologist to create and extend awareness of nematode problems, in a moderated and pragmatic manner that encapsulates the issue realistically – without exaggeration.

Anticipating Future Challenges to Nematology

In previous versions of this chapter (Luc *et al.*, 1990, 2005) and in the reviews by Taylor (1976),

De Waele and Elsen (2007), Ciancio (2015) and Talwana *et al.* (2016), factors important to the practical management of nematological problems in the tropics were discussed and elaborated on. Emphasis was placed on the attention that needed to be taken to make practical management applicable for the tropics, including: the need for taxonomy and diagnostics, yield loss studies, extension, disease complexes and multi-disciplinary research. In this chapter, we have discussed most of these issues with present-day knowledge.

To generate discussion and stimulate our thought processes with regards to the future of tropical nematology, we decided to try to anticipate the status of agriculture and nematology in the tropics in the distant future (Tables 1.2 and 1.3). We use the concept of anticipation that involves natural, formal and social systems to develop intentionally or unintentionally ideas of a future so that we can act effectively in the present (Poli, 2014). Hopefully, the thought-provoking statements made on the future of agriculture and nematology will help us to foresee the problems tropical nematology will face as time advances from 2017 to 2050 and 2100. Our goal is to stimulate discussion on scientific advancement in nematology as it relates to changes in agriculture over time. The 83-year timespan in the tables is approximately three scientist generations at a major research institution, or that of our successor's successor and beyond.

The changes taking place in the tropical regions of the world now and in the future are and will be immense: increasing population densities, mass urbanization, migration, severe climate change impacts, expanding levels of soil degradation, loss of soil fertility, as well as increasing levels of hunger and malnutrition in many tropical countries. These factors will affect how tropical agriculture and nematology will be organized and directed in the future. These interacting factors will have a life-changing impact on human migration and food production. What is the role of nematology in the future?

If we consider the anticipated changes affecting agriculture and nematology in the future as an outcome of overpopulation, degradation of agricultural soils, climate change and a lack of adequate pest and disease management, nematology in the tropics will face major challenges in the future. Some of the challenges that need

to be considered for research in the future include the following:

- Determine the impact of nematodes on crop yield in smallholder agriculture.
- Clarify the importance of multispecies complexes in crop loss.
- Examine the importance of nematode–fungal root rot interactions.
- Determine nematode impact in mixed cropping systems.
- Improve the understanding of nematode–abiotic stress interactions on yield.
- Work with breeders to develop resistant/tolerant cultivars.
- Introduce conservation agriculture to improve soil and crop resiliency.
- Expand research on biological control and soil antagonistic potential.
- Study the impact of nematode–abiotic stress interactions on root architecture.
- Expand diagnostic tools to all economically important tropical nematodes.
- Monitor crops for emerging nematode problems.
- Determine temperature distribution patterns.
- Expand research to include endo- and ectoparasitic species.
- Strengthen quarantine through advanced training.
- Improve nematode control on large-holder farms to attain maximum yield.
- Transfer proven management techniques to smallholders.
- Develop management programmes to prevent loss of resistance.
- Educate on the safe use of nematicides.
- Promote targeted management technologies, such as seed treatment.
- Advance the use of remote sensing.
- Improve knowledge transfer to all farmers with Internet technology.
- Promote scientific cooperation between major centres of nematology.
- Collaborate in attracting funding for research conducted in the tropics.
- Work in the present but anticipate the future.

We have outlined some of the difficulties facing nematology in the tropics and listed the challenges we believe are important. We wish to emphasize that none of the anticipated

Table 1.2. Anticipation of future developments in agriculture in the tropics from 2017 to 2050 and 2100.

Status of Agriculture		
Current	2050	2100
1. Human population reaches 7 billion	1. Human population reaches 9.5 billion	1. Human population exceeds 11.5 billion
2. Atmospheric temperature +1°C	2. Atmospheric temperature +2°C	2. Atmospheric temperature +4°C
3. Drought and high temperature reduce yields in dryland farming sporadically	3. Droughts and high temperature repeatedly limit production in semiarid and tropical-dry regions	3. Drought and high temperatures prevents crop production in many semiarid regions
4. Production on largescale farms near stagnating	4. Production on large farms slightly improved	4. Production on large farms maximized
5. Yields very low smallholder farms in tropics	5. Yields improve marginally in smallholder farms in the tropics	5. Smallholder farm yields increase greatly with good farming practices
6. Smallholder farms in tropics decreasing in size < than 1–2 ha	6. Small farm size begin to increase in size	6. Small farm size expands greatly with new land reform laws
7. Smallholder farm yield <80% of largescale farms	7. Smallholder farms yields begin to improve	7. Smallholder farm yields improve significantly
8. Land reform often absent	8. Land reform being adapted	8. Land reform effective
9. Input subsidies lacking	9. Input subsidies stimulate yield	9. Inputs increase production levels
10. Soil degradation extreme	10. Soil quality improving slowly	10. Soil quality significantly improved
11. World food reserves < 60 days	11. World food reserves recover	11. World food reserves at all time high
12. Food shortages common	12. Food shortages reduced	12. Food shortages a thing of the past
13. Hunger common in many tropical countries	13. Hunger levels reduced as production improves	13. Hunger no longer a major problem worldwide
14. Migration to mega cities accelerates	14. Migration to mega-cities shifts to med-size cities and slows	14. Migration stabilizes toward midsize cities
15. Food production needs to rise 2% annually to feed populations	15. Food production increases steadily	15. Cooperatives common and aiding yield stability
16. Rising sea levels predicted irrigated lowland rice affected	16. Rising sea levels promote upland rice	16. Upland rice production predominates
17. Demand for meat increasing worldwide	17. Demand for meat beginning to stagnate	17. Demand for meat decreased significantly
18. Lack of horticultural products affect nutrition	18. Cooperatives and peri-urban horticulture increases	18. Demand for high quality horticultural products rises
19. Food production per capita at all time low	19. Food production per capita adequate	19. Food production per capita keeping pace with human growth

Table 1.3. Anticipation of future developments in nematology in the tropics from 2017 to 2050 and 2100.

Nematology		
Current	2050	2100
1. Crop yield in semiarid and tropical-dry zones anticipated	1. Yield losses in these zones increases significantly	1. Some of these areas no longer productive for dry land farming
2. Climate change impact on nematode damage expected	2. Warmer climates responsible for rise in nematode damage	2. Nematode generations/season, damage increases significantly
3. Yield loss underestimated in smallholder farms –SHF	3. Yield losses measured in SHF and nematode damage recognized	3. Yield increases with site- and crop-specific IPM management
4. Nematode impact on root architecture underestimated	4. Nematode impact on root structure recognized by breeders	4. Cultivars with nematode and drought resistance available
5. SHF do not recognize nematode damage in mixed-crop systems	5. Nematode damage on SHF recognized, control lacking	5. Management tools for major nematode problems available
6. Multi-species nematode impact not researched	6. Molecular tools improve multi-species research	6. Resistant/tolerant cultivars for multi-species problems developed
7. Breeders still lack sources of resistance to many nematodes	7. Breeders develop cultivars with drought/ nematode resistance	7. Nematode/drought resistant cultivars are available
8. Low soil fertility-nematode interactions poorly researched	8. Soil fertility-nematode complexity investigated	8. Management for soil fertility - nematode complexity available
9. SHF mixed cropping systems mask nematode damage	9. SHF moves to fewer crops, nematode damage increases	9. Near monoculture conditions promote nematodes damage
10. New emerging nematode problems going unnoticed	10. Emerging nematode problems detected and studied	10. New nematode problems addressed with IPM
11. Nematode interactions with root pathogens poorly understood	11. Complex interactions research still poorly studied	11. Multispecies and complex interactions well researched
12. Diagnostic identification tools lacking for many species	12. Diagnostic identification available for important species	12. Diagnostic tools in available for extension nematologists
13. Agricultural science at universities in decline	13. Importance of applied agricultural science recognized	13. Agricultural universities develop major hubs of expertise
14. Nematology extension virtually non-existent	14. Nematology extension expanding, internet based	14. Nematology extension reaches all farmers digitally
15. Applied nematology positions on a decline, funding poor	15. Applied nematology positions increase, funding increases	15. Applied nematology is given high priority, strong funding
16. IPM limited to largescale farms and monocrop systems	16. IPM targets major pests on large and SHF systems	16. IPM is farmer driven and site specific and extension supported
17. Invasive nematode spreading understaffed quarantine	17. Invasive nematodes impacting production, quarantine improved	17. Invasive spread reduced, quarantine effective in tropics
18. Minimum tillage on smallholder farms absent, organic matter low	18. Minimum tillage increases organic matter	18. Minimum tillage widespread and improves eco-services
19. Antagonistic and ecosystem services not used in IPM	19. Effective measurement of eco-system potential attained	19. Antagonistic potential and ecosystem resiliency part of IPM
20. Presence of resistance breaking populations unknown	20. Resistant breaking populations in important species identified	20. New resistant cultivars detected and available
21. Genetically modified nematode resistant crops unavailable	21. Genetically modified crops with nematode resistance a reality	21. Genetically modified resistance available for site-specific use
22. Chemical & biological nematicides in development	22. Chemical/biological nematicide synergism used in IPM	22. New forms of nematicides and innovative applications developed
23. Remote sensing well researched but limited adaption in field	23. Remote sensing used for site specific IPM, robots use tested	23. Remote, micro-sensors and robots used in large scale IPM
24. SHF what is the unseen enemy?	24. SHF recognize the unseen enemy	24. IPM of Unseen enemy practiced

problems facing agriculture in the tropics in the future are insurmountable with the appropriate effort, expertise and backing. You will notice, reading through the various chapters, that a great deal of new knowledge on the importance of nematodes as plant parasites, and more relevantly the successes in their management, has been accumulated by nematologists

since the printing of the second edition. We estimate that over 10,000 research articles have been published on tropical nematodes affecting crops since the last edition of this book, and many of these have been integrated in the chapters in this book. We anticipate, and look forward to, a bright and successful future for nematology.

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2 Identification, Morphology and Biology of Plant Parasitic Nematodes*

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Nematodes successfully colonize a greater variety of habitats than any other group of multicellular animals. Many species are free-living, feeding on bacteria or fungal spores, whereas others are predatory or parasitic in habit. The predatory forms feed on many groups of soil invertebrates, including other nematodes, while the parasitic forms utilize as hosts a wide variety of algae, fungi, higher plants, invertebrates, etc. However, despite such ecological diversity, nematodes are surprisingly similar in their structure.

This chapter starts with a brief, simplified account of the basic morphology, anatomy and bionomics of plant parasitic nematodes, followed by illustrated descriptions that concentrate on the diagnostic features of the most commonly occurring and/or most important plant parasitic genera referred to in the following chapters.

General information on nematode morphology and biology can be found in Dropkin (1980), Nickle (1991), Geraert (2006), Manzanilla-López and Marbán Mendoza (2012), Perry and Moens (2013), and Schmidt-Raesä (2014). In addition, excellent illustrated descriptions of plant parasitic nematodes, together with data on biology and classification, can be found in Nickle (1991), Siddiqi (2000), Castillo and

Vovlas (2005, 2007), Geraert (2008, 2010, 2011, 2013), Subbotin *et al.* (2010a, b), Manzanilla-López and Marbán Mendoza (2012), Subbotin and Chitambar (2014) and Ghaderi *et al.* (2016).

Morphology of Plant Parasitic Nematodes

Plant parasitic nematodes almost invariably bear a mouth spear for penetrating plant cells, a feature that distinguishes them from the majority of other soil nematodes. It should be borne in mind, however, that non-phytoparasitic dorylaims also have a spear, as do many mycophagous, predatory or insect parasitic nematodes. The spear has evolved independently in each of the three major groups of plant parasitic nematodes. In the Tylenchina (tylenchs) and Aphelenchoididae (aphelenchs), the spear is also known as the **stylet**; in the Longidoridae (Dorylaimida), it is called the **odontostyle**; and in the Trichodoridae (Triplonchida), the **onchiostyle**. The tylenchs are the most speciose and economically important group of plant parasitic nematodes worldwide, and will be dealt with in greater detail.

*A revision of the chapter by D.J. Hunt, M. Luc and R.H. Manzanilla-López in the 2nd edition.

Tylenchs and aphelenchs (Fig. 2.1a–j)¹

Tylenchs and aphelenchs are typically vermiform and bilaterally symmetrical animals that usually range from 0.2 to 1 mm in length. In some genera, the female loses the vermiform habit, becoming obese, even globose, in form.

The **labial region**, when seen *en face* (Fig. 2.1c), is typically hexaradiate and has a central orifice, the mouth, through which the hollow stylet is

protruded. Various sensory structures, including the **amphidial apertures**, occur on the labial region, which is often transversely annulated and usually separated from the body by a constriction. Internally, the labial region contains a sclerotized **framework** (or skeleton) that supports the structure and acts as an anterior attachment point for the stylet protractor muscles.

The body is enclosed in a cuticle, which is usually transversely annulated (h1) and may be

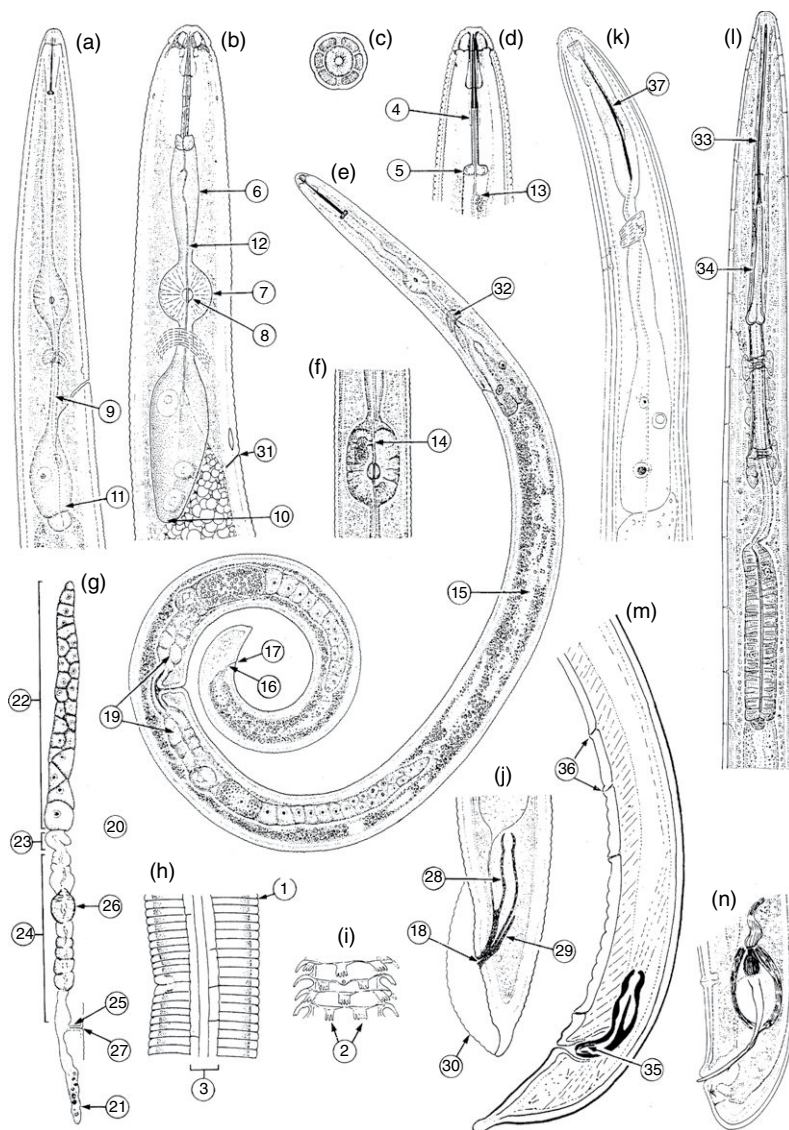


Fig. 2.1. Major diagnostic features of plant parasitic nematodes. Line drawings are for illustrative purposes only and are not to scale. Numbers in circles refer to structures mentioned in the text.

ornamented with a variety of processes in the criconematid forms (i2). Longitudinal ridges occur in some species. Beneath the cuticle are the **hypodermis** and the longitudinal muscles, which are attached to four **chords** – longitudinal thickenings of the cuticle and hypodermis. The lateral chords are better developed than the ventral and dorsal ones and correspond externally to the **lateral field**, which is marked by a number of **longitudinal lines** (h3) or **incisures**, the region between two incisures being known as a **band** or **ridge**. The central cavity of the nematode, the **pseudocoelom**, contains a viscous fluid that acts as a **hydrostatic skeleton**. Suspended within the fluid are the three major organ systems – digestive, reproductive and excretory.

The digestive system comprises the **stylet**, **pharynx**, **intestine** and **rectum**. The stylet (d4) is a protrusible cuticular tube, pointed anteriorly and with a subterminal aperture. It consists of an anterior conus attached posteriorly to a more or less cylindrical shaft, the latter generally swelling posteriorly to form three **basal knobs** (d5). Protractor muscles are attached to the knobs and extend anteriorly to the labial (or cephalic) skeleton.

The pharynx comprises a narrow cylinder or procorpus (b6), which expands to form the **median bulb** (b7), a muscular swelling containing refringent valve plates (b8), before narrowing to the **isthmus** (a9) and then expanding into a glandular portion (b10, a11). There are three, one dorsal and two subventral, pharyngeal glands that may form a bulb-like structure (a11) abutting the intestine, or may be extended into a lobe overlapping the intestine (b10). Between the stylet base and the pharyngo-intestinal junction runs a central tube, the **pharyngeal lumen** (b12), through which glandular secretions and food pass. In tylenchs, the dorsal pharyngeal gland opens into the pharyngeal lumen near the stylet base (d13), the two subventral glands opening within the median bulb, whereas in aphelenchs all three glands open within the median bulb (f14). The **intestine** (e15) is a largely undifferentiated tube, which opens via the **rectum** (e16) at the **anus** (e17) or, in adult males, the **cloaca/cloacal aperture** (j18). In the males of certain genera, the digestive system is degenerate and non-functional.

The reproductive system in both sexes is tubular. The female genital system may be composed of two (e19), usually opposed, branches

(**didelphic**) or reduced to a single branch (**monodelphic**). In monodelphy (g20), the posterior branch may be reduced to a **post-uterine sac** (g21) or be entirely absent, the other branch running anteriorly (**monoprodelfic**). Each branch has four major parts: **ovary** (g22), **oviduct** (g23), **uterus** (g24) and **vagina** (g25). There may also be a **spermatheca** (g26), a specialized uterine structure for storing sperm. The vagina opens to the exterior via the **vulva** (g27), a ventrally situated transverse slit in the middle or posterior section of the body. The male reproductive system is less variable. The single genital tube consists of a **testis**, **seminal vesicle** and **vas deferens** opening to the exterior with the intestine via a common orifice, the cloacal aperture (j18). The copulatory organ consists of the paired **spicules** (j28) with a guiding piece, the **gubernaculum** (j29). The protrusible spicules are heavily cuticularized and serve to open the female vulva and channel sperm. The male tail often has cuticular expansions, the caudal alae (j30) or **bursa**, to assist in copulation. The excretory system consists of a uninucleate **gland cell** connected via an **excretory canal** to the ventrally situated **excretory pore** (b31). This pore is usually in the pharyngeal region, but may be located far more posteriorly (e.g. *Tylenchulus*).

The nervous system consists of the **nerve ring** (e32), a circumpharyngeal (sometimes circumintestinal) commissure, plus a network of nerves connected to body organs and various sensory structures. These sense organs are mostly in the labial region (**sensilla** and **amphids**), the pharyngeal region (**cephalids**, **deirids**, **hemizonid** and **hemizonion**) and on the tail (**phas-mids** and **caudalids**).

Longidoridae (Fig. 2.1l and m)

Compared with tylenchs, longidorids are much longer nematodes and range from 0.9 to over 12 mm in size. The cuticle is smooth, and lateral fields are absent. The protrusible spear has a different origin from that of the tylenchs and is more properly called an odontostylet. It may be up to 300 µm long and consists of a needle-like **odontostyle** (l33) attached posteriorly to a cuticular extension, the **odontophore** (l34). A cuticularized **guiding ring** is located around the odontostyle. The pharynx consists of a narrow anterior section and a posterior cylindroid expansion, which

is both muscular and glandular. The female reproductive system is either didelphic or monodelphic; in the latter case, the anterior branch regresses and only the posterior branch remains (**opisthodelphic**). The male spicules are well developed and have lateral guiding pieces (m35). There is no gubernaculum or bursa, but a ventral series of sensory supplements (m36) run anteriorly from the cloacal aperture. Some morphological features of tylenchs, such as excretory pore, phasmids, deirids and cephalids, are missing, whereas numerous somatic cuticular pores are present along the body.

Trichodoridae (Fig. 2.1k and n)

Trichodorids are rather plump, cigar-shaped nematodes, about 0.5–1.1 mm long and with a bluntly rounded labial region and tail. The cuticle is smooth and may swell enormously under the influence of acidic fixation. The curved spear is actually a mural tooth, and is properly referred to as an **onchiostyle** (k37). The pharynx comprises a narrow cylindrical anterior section that swells gradually into a posterior bulboid expansion. The female genital system is usually didelphic, very exceptionally monodelphic. The male spicules are curved slightly and a weak bursa may be present. Ventral supplements occur.

Novel Approaches to Identification

Phylogenetic analysis of 18S ribosomal DNA sequences indicates a close relationship between arthropods, nematodes and all other moulting phyla, which are included in the clade Ecdysozoa (Aguinaldo *et al.*, 1997; Dunn *et al.*, 2008). Molecular methodologies in nematode identification and systematics have advanced tremendously in the past decade or so (see De Ley and Blaxter, 2002; van Megen *et al.*, 2009; see Chapter 4, this volume). Although used widely in systematics and phylogenetic studies, molecular techniques are also applied increasingly to species identification, particularly so in morphologically conserved and/or speciose groups, such as the cyst and root knot nematodes, *Bursaphelenchus*, *Xiphinema*, or in genera where complexes of cryptic species are known to occur (e.g. *Pratylenchus*, *Meloidogyne*, *Ditylenchus*, *Helicotylenchus*). Increasing attention is also being paid to other intractable taxa,

including the anguinids. In groups such as the heteroderids and meloidogynids, isozyme methodologies remain an important diagnostic tool. New molecular tools, such as metabarcoding using next-generation sequencing, could help to identify, at least to some degree (species or genus level), all nematodes in a sample if their sequences are available in open databases. These powerful techniques, however, have problems that must be taken into account; for example, short-length fragments, bias by primer matching, intra-individual variability of rRNA genes, or bioinformatics knowledge. However, there have been some interesting attempts at understanding this diversity (Porazinska *et al.*, 2012; Dell'Anno *et al.*, 2015), and in future, as the sequenced fragment length increases with improvements in technology, accuracy will also increase.

Cryptic Speciation

The integration of morphological and molecular studies has shown the presence of cryptic species in plant parasitic nematodes. Bickford *et al.* (2006) defines a 'cryptic species' as two or more distinct species (species complex) that are classified erroneously (and 'hidden') under a single species name. Species delimitation in nematodes uses relatively few anatomical and morphological characters, and these characters are often highly conserved between taxa. Increasingly, morphological identification must be supported, or even supplanted, by molecular markers. The increase in the number of species with molecular sequences or genomes deposited in open online databases, and the use of topotypes for molecular reference markers, will be of increasing utility.

Bionomics of Plant Parasitic Nematodes

Reproduction and development

Reproduction is usually either amphimictic (separate males and females) or parthenogenetic (males absent, very rare or non-functional), although hermaphroditism may also occur. Eggs may be laid singly or stuck together in masses in a gelatinous matrix secreted by the female. Such egg masses are associated with species where the

females swell and become sedentary, although some obese genera retain all the eggs within the body, the cuticle tanning on the death of the female to form a tough cyst. Egg sacs and cysts serve both to protect and to disperse eggs.

Nematodes typically have four, exceptionally three (as in some longidorids), juvenile stages between the egg and the adult, the intervening moults facilitating an increase in size. In tylenchs and aphelenchs, the first-stage juvenile, or J1, moults to the second-stage juvenile (J2) within the egg, but in longidorids and trichodorids, it is the J1 that hatches. Depending on the parasitic habit of the nematode, hatched juveniles may be found in the soil, but can also occur inside the roots, either post-invasion or from eggs laid and retained within the root tissues.

Environmental conditions

Although occupying many different ecological niches, nematodes are essentially aquatic animals. Plant parasitic nematodes require at least a film of water to enable locomotion and, as all species spend a greater or lesser proportion of their life in soil, its water content is a primary ecological factor. Although many species die in dry soils, others may survive in an anhydrobiotic state. Conversely, too much soil water may result in a lethal oxygen deficit, although certain genera (e.g. *Hirschmanniella*) thrive under such conditions.

Soil temperature is rarely a particularly important factor as it tends to remain reasonably stable in a given environment. Some tropical nematodes survive soil temperatures of 50°C, provided that sufficient time is available for them to enter anhydrobiosis.

Soil structure is influential as pore size affects the ease with which nematodes can move through the soil interstices. In general, sandy soils provide the best environment, soils with a high clay content or those with an excessively open texture inhibiting movement. However, saturated clay soils can be colonized successfully by certain specialized nematodes, including *Hirschmanniella* and some *Paralongidorus*. Soil pH may affect nematodes, but few data are available for tropical and subtropical species.

The maxim that 'where a plant is able to live, a nematode is able to attack it' is a good one. Nematodes are even able to attack the aerial

parts of plants provided that the humidity is high enough to facilitate movement. Such conditions are provided in flooded rice fields, where foliar species, such as *Aphelenchoides besseyi* and *Ditylenchus angustus*, can be devastating. Some *Bursaphelenchus* species, vectored by wood-boring beetles, directly attack the trunk of palms or pines. Other nematodes, such as some *Hirschmanniella* and *Halenchus* spp., attack marine algae and can live in seawater.

Hatching, host location and penetration

The eggs of many plant parasitic nematodes are deposited singly, either in the soil or within the plant tissues. Provided that other factors are favourable, they usually hatch irrespective of the presence of a host plant.

In the more advanced parasites, however, the eggs may be embedded in a gelatinous matrix to form an egg mass (e.g. *Meloidogyne*, *Nacobbus*) or retained within the swollen female body, the cuticle of which tans to form a protective cyst (e.g. *Heterodera* and *Globodera*). Egg hatch in cyst nematodes is stimulated by root exudates from the host, a requirement that implies a restricted host range. Nematodes are attracted to plant roots by a variety of factors that have yet to be fully elucidated. Such attractants can operate over considerable distances – up to 1 m, for example, in *Meloidogyne*.

There are three main types of parasitism (Fig. 2.2):

- 1. Ectoparasitic** – the nematode remains in the soil and does not enter the plant tissues. It feeds by using the stylet to puncture plant cells; the longer the stylet, the deeper it can feed. The majority of ectoparasitic species remain motile, whereas some others, e.g. *Cacopaurus*, are permanently attached to the root by the stylet, which is deeply embedded in the plant tissue.
- 2. Semi-endoparasitic** – only the anterior part of the nematode penetrates the root, the posterior section remaining in the soil phase.
- 3. Endoparasitic** – the entire nematode penetrates the root. **Migratory endoparasites** retain their mobility and have no fixed feeding site within the plant tissue, whereas **sedentary endoparasites** have a fixed feeding site and induce a sophisticated trophic system of nurse cells or syncytia, thus allowing them to become obese and thereby lose their mobility.

The above categories are not mutually exclusive as some genera may, depending on the host, be either semi-endoparasitic or migratory ectoparasitic, e.g. *Helicotylenchus*, while some sedentary parasites have only the anterior body embedded in the root (= **sedentary semi-endoparasites**), e.g. *Rotylenchulus* and *Tylenchulus*.

In *Meloidogyne* and *Heterodera/Globodera*, the J2 is the infective stage, but in ectoparasites and most migratory endoparasites any vermiform stage may feed on, or penetrate, the root (Fig. 2.2). Rarely, as in *Rotylenchulus*, the immature female

is the infective stage, the non-feeding juveniles and males remaining in the soil.

Host reactions

As ectoparasites, e.g. *Tylenchorhynchus*, do not enter the plant, the damage they cause is usually limited to necrosis of the superficial cells able to be penetrated by their short stylet. However, species with longer stylets, such as *Xiphinema* or *Hemicycliophora*, can penetrate the tissues much

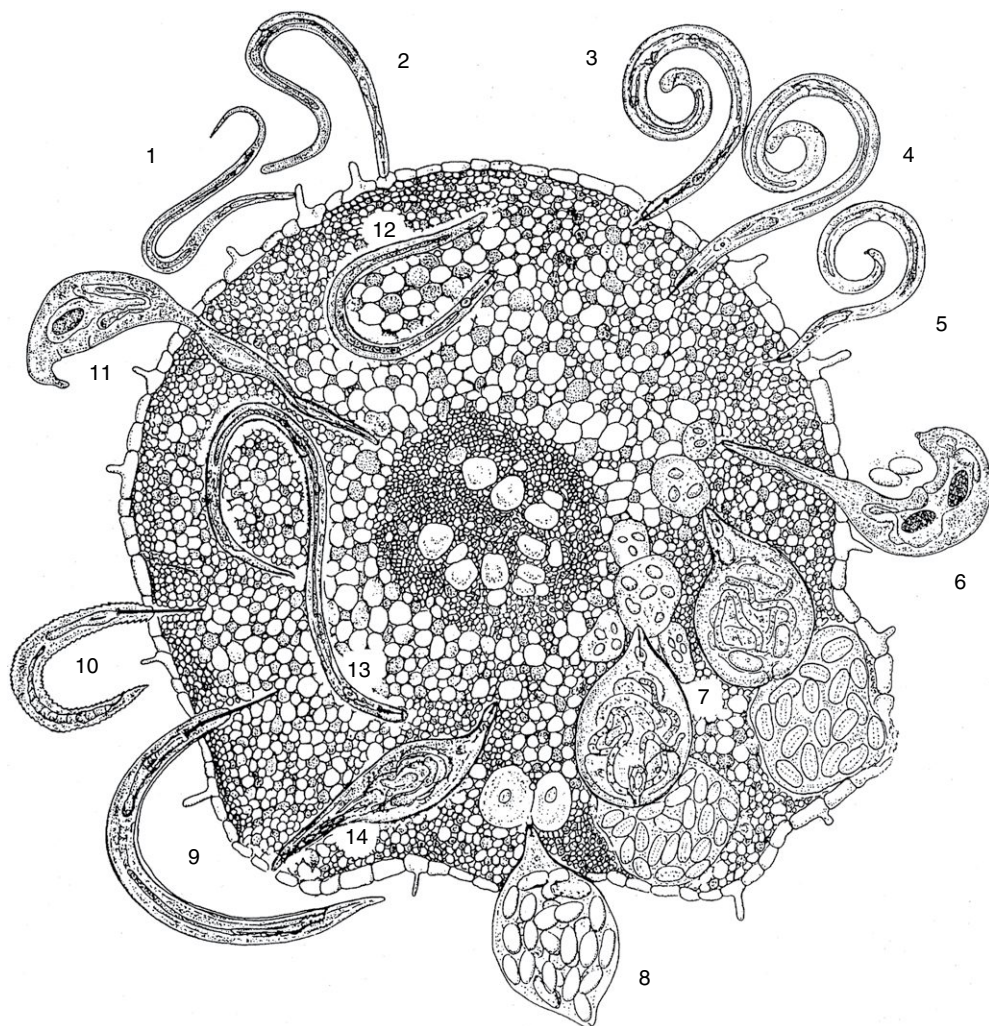


Fig. 2.2. Diagrammatic presentation of various types of tylenchid nematode feeding on root tissue.

1. *Ditylenchus*. 2. *Tylenchorhynchus*. 3. *Rotylenchus*. 4. *Hoplolaimus*. 5. *Helicotylenchus*. 6. *Rotylenchulus*. 7. *Meloidogyne*. 8. *Heterodera*. 9. *Hemicycliophora*. 10. *Criconemoides/Mesocriconema*. 11. *Tylenchulus*. 12. *Pratylenchus*. 13. *Hirschmanniella*. 14. *Nacobbus*. (From Siddiqi, 2000.)

more deeply, thereby killing more cells. Such nematodes tend to feed on meristematic tissue near the root tips, the concomitant damage resulting in galling or hooked roots and, if the growing point is destroyed, stimulating secondary root proliferation behind the tip.

Endoparasites not only kill the cells they feed on but also, by burrowing through the root tissues, cause extensive destruction, leading to cavitation, secondary infection and rotting roots. Successive generations of nematodes compound the damage, and it is not surprising that some of the most pathogenic nematodes belong to this group (*Pratylenchus*, *Radopholus* and *Hirschmanniella*).

Sedentary endoparasites have a sophisticated relationship with the host that involves transformation of root cells into a trophic system of nurse or transfer cells. The trophic system functions as a nutrient sink so that the sedentary nematode is provided with a copious supply of nutrients, thus enabling it to increase enormously in size and thereby produce more eggs. In *Meloidogyne*, proliferation of root cells is also incited, thus causing the characteristic galls.

Plants with the root system damaged by nematodes often show above-ground symptoms such as stunting, chlorosis, wilting, early senescence and reduced yield. These symptoms are a direct result of the impaired ability of the root system to deliver water and nutrients, and thus may be confused with similar symptoms resulting from poor soil conditions and/or nutrient deficiencies.

The exact ways in which nematodes affect plants have yet to be fully elucidated, and besides impairing root function by physical damage, toxins may also be involved. An interesting case is 'Ontario peach decline', where a very low population of *Pratylenchus* can kill young trees. The nematodes metabolize the sugar part of cyanosides in the plant tissue and thus liberate the CNH radical, which is highly toxic to the tree. Nematodes secrete effectors that may interfere with various pathways in the plant, thereby facilitating the migration of the nematode through the plant tissues, procurement of food sources, and avoiding plant defence mechanisms.

In nematology, a number of terms are used to describe the interrelationships of host and parasite. Plants can be divided into **hosts** or

non-hosts, depending on whether nematode reproduction occurs. Non-hosts may be **immune**, i.e. no nematode penetration or reproduction, or **resistant**, i.e. allowing nematode penetration and a varying degree of parasitism, but not reproduction. Host plants are non-resistant or susceptible and can be good or poor hosts, depending on whether reproduction is high or low. Susceptible plants, which support the lowest levels of reproduction within a data set, have been referred to as partially resistant or even, in some cases (in an agronomical concept), as 'resistant'. Some resistant plants are used as 'trap crops' to attract the nematodes in the soil before sowing a crop susceptible to the nematode in question.

Variations in the ability of nematodes to reproduce on given plant species or cultivars are of great agricultural significance. Nematode populations distinguished by their ability or inability to reproduce on designated plant species are known as **host races**. **Pathotypes** are variants of a host race or species, which are distinguished by their ability to reproduce on a designated host plant genotype (e.g. cultivar, line, etc.).

Tolerance refers to the amount of damage caused by the nematode to the plant and should not be confused with resistance (q.v.). A **tolerant host** suffers little damage even when heavily infected, while an **intolerant host** may be severely damaged, even if only lightly infested.

Survival

Nematodes respond to different environmental stimuli through physiological strategies, sometimes assisting survival by arresting development, a condition known as diapause or quiescence. In the absence of a live host, nematodes may survive in the soil or in plant residues. Provided that the environment dries slowly, many nematodes are able to enter a reversible anhydrobiotic state, when they are less susceptible to desiccation, temperature extremes and chemicals. In a number of genera, the eggs are the survival stage, being protected either in a gelatinous matrix (*Meloidogyne*, *Tylenchulus* and *Rotylenchulus*) or within the hardened cyst-like body of the dead female (*Heterodera* and *Globodera*). In the latter case, infective J2 nematodes may not hatch for several years after being laid. Anhydrobiosis is probably more common in tropical and

subtropical areas than is currently realized and enables the organism to survive the dry season and also to nullify some non-chemical control methods, such as dry fallow. The record for longevity in the anhydrobiotic state is held by seed nematodes, such as *Anguina*, which have been recorded surviving for 39 years. A practical consequence of anhydrobiosis is that extraction from dry soil requires a sufficient period of soaking for the nematodes to absorb water and thereby attain the active state.

Identification of the Major Genera

This section is intended to serve as a basic guide to the identification of the major parasitic genera found in tropical and subtropical agriculture. Each generic diagnosis has the major differential characters printed in bold. Genera are arranged according to systematic position (Table 2.1) rather than trophism. A full list of scientific authorities is given in the Appendix.

Table 2.1. Simplified outline classification.

Order/suborder/superfamily	Family	Genus	Page
TYLENCHOMORPHA			
Aphelenchoidoidea	Aphelenchoididae	<i>Aphelenchoides</i>	28
		<i>Bursaphelenchus</i>	29
Tylenchina			
Tylenchoidea			
	Anguinidae	<i>Ditylenchus</i>	31
		<i>Anguina</i> ^a	32
	Belonolaimidae	<i>Tylenchorhynchus</i>	33
	Pratylenchidae	<i>Pratylenchus</i>	35
		<i>Hirschmanniella</i>	37
		<i>Radopholus</i>	38
		<i>Nacobbus</i> ^a	40
	Hoplolaimidae	<i>Helicotylenchus</i>	41
		<i>Hoplolaimus</i>	43
		<i>Scutellonema</i>	43
		<i>Aorolaimus</i>	43
		<i>Aphasmatylenchus</i>	45
		<i>Rotylenchulus</i> ^a	46
	Heteroderidae	<i>Heterodera</i> ^a	48
		<i>Globodera</i> ^a	49
	Meloidogynidae	<i>Meloidogyne</i> ^a	49
Criconematoidea	Criconematidae	<i>Criconemoides</i>	51
		<i>Mesocriconema</i>	51
		<i>Hemicycliophora</i>	52
		<i>Hemicriconemoides</i>	54
	Tylenchulidae	<i>Tylenchulus</i> ^a	54
DORYLAIMIDA			
Dorylaimina			
Longidoroidea	Longidoridae	<i>Xiphinema</i>	56
		<i>Longidorus</i>	56
		<i>Paralongidorus</i>	56
TRIPLONCHIDA			
Diphtherophorina			
Trichodoroidea	Trichodoridae	<i>Trichodorus</i>	58
		<i>Paratrichodorus</i>	59
		<i>Nanidorus</i>	59

Note: ^aGenera with obese sedentary females.

***Aphelenchoides* Fischer, 1894**
(Aphelenchoididae) (Fig. 2.3)

MORPHOLOGY: small to medium sized (0.4–1.2 mm), slender nematodes. **Females** die straight or ventrally arcuate on heat relaxation, while

the male tail curls ventrally to produce a 'walking-stick' shape. Labial region weakly sclerotized; stylet weak, with or without basal swellings. **Pharyngeal bulb well developed, spherical to rounded-rectangular in shape and more or less filling the body**

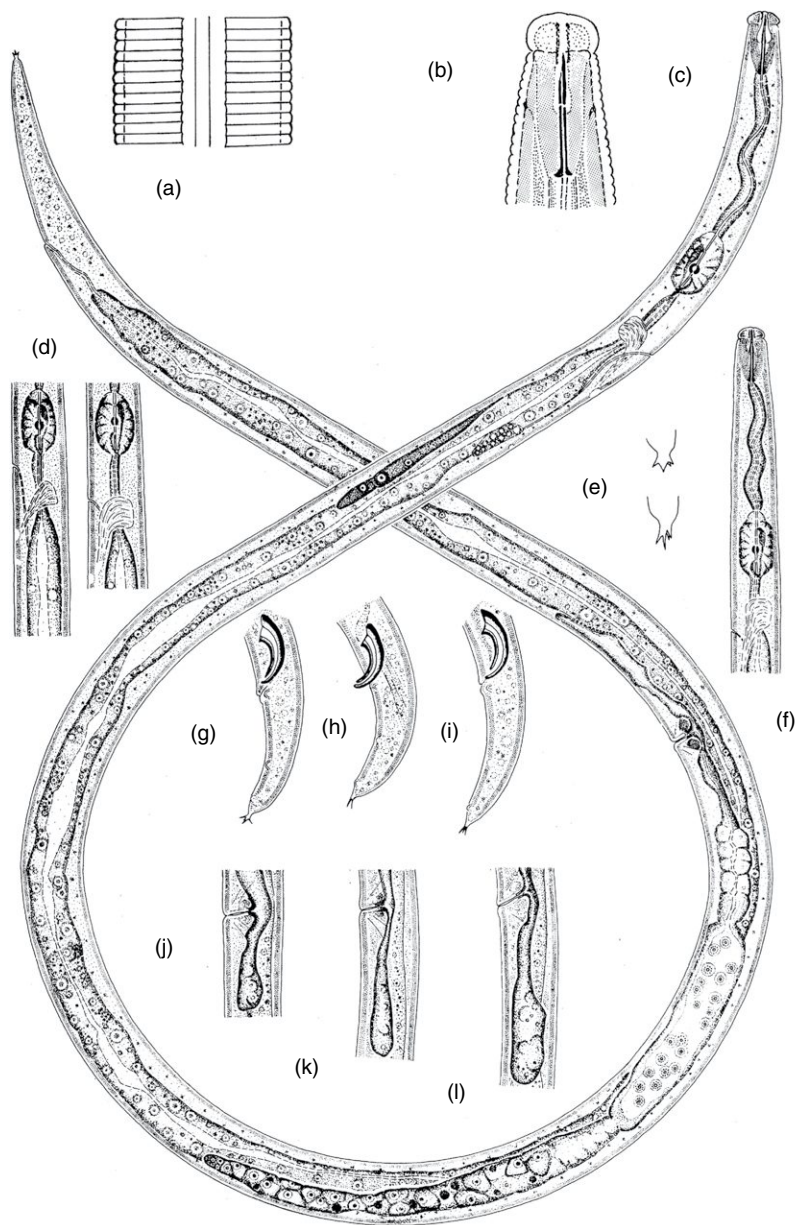


Fig. 2.3. *Aphelenchoides besseyi* (a) lateral field; (b) labial region; (c) entire female; (d) median bulb and excretory pore position; (e) female tail tips; (f) pharyngeal region; (g–i) male tail region; (j–l) post-vulval sac. Line drawings are for illustrative purposes only and are not to scale.

diameter. Dorsal pharyngeal gland duct opening within bulb, just anterior to the valve plates. Pharyngeal gland lobe overlapping intestine dorsally. Female: vulva posterior (60–75%); genital tract single, anteriorly directed. Tail medium conoid, with or without terminal mucron(s). Male: tail medium conoid, spicules well developed, thorn shaped. No bursa.

BIOLOGY: ectoparasitic on leaves, stems and other parts of higher plants. Most species can also be cultured readily on various fungal hyphae. *A. besseyi* can withstand desiccation for

several years. The life cycle is rapid and may be completed in as little as a week, depending on temperature.

MAJOR SPECIES: *Aphelenchoides* is a very speciose genus, the majority being fungal feeders. Several species, however, are also important phytoparasites, e.g. *A. arachidis*, *A. besseyi*, *A. ensete*, *A. fragariae* and *A. ritzemabosi*.

DISTRIBUTION: *A. arachidis* is only currently recorded from groundnut (peanut) in northern Nigeria and South Africa, but the other species are well distributed, with *A. besseyi* being found in most rice-growing areas.

Useful literature

- Hunt, D.J. (1993) *Aphelenchida, Longidoridae and Trichodoridae: Their Systematics and Bionomics*. CAB International, Wallingford, UK.
- Hunt, D.J. (2008) A checklist of the Aphelenchoidea (Nematoda: Tylenchina). *Journal of Nematode Morphology and Systematics* 10, 99–135.
- Kanzaki, N. and Giblin-Davis, R.M. (2012) Aphelenchoidea. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 161–208.
- Shahina, F. (1996) A diagnostic compendium of the genus *Aphelenchoides* Fischer, 1894 (Nematoda: Aphelenchida) with some new records of the group from Pakistan. *Pakistan Journal of Nematology* 14, 1–32.

***Bursaphelenchus* Fuchs, 1937 (Aphelenchoididae) = *Rhadinaphelenchus* J.B. Goodey, 1960 (Fig. 2.4)**

MORPHOLOGY: the genus is similar in general respects to *Aphelenchoides*, although the male has **differently shaped spicules and cuticular alae (the 'bursa') on the tail tip**. In *Bursaphelenchus cocophilus*, **both sexes are very slender (body length/body diameter = about 100)**. In addition, **the female has an extremely long post-vulval sac, very long, slightly tapering tail with a rounded tip, and a vulval flap**. The male tail tip bears a small cuticular flap (the 'bursa'), which is most easily visible in ventral view. **Dorsal limb of spicule elongate.**

BIOLOGY: mostly ectophoretic associates of various insects, including Coleoptera and Hymenoptera. There are two major phytoparasitic species, both being vectored by wood-boring insects: *Bursaphelenchus xylophilus*, which attacks pine

trees; and *B. cocophilus* (formerly known as *Rhadinaphelenchus cocophilus*), which is parasitic in the stem of coconut palms, of which 10 g of tissue may contain 50,000 nematodes. *B. cocophilus* may also be found in cortical tissues of coconut roots. Infection often causes the development of a red or orange-red ring of tissue within the stem (hence the common name of 'red ring nematode'). The nematode is vectored by the *Rhynchophorus* palm weevil during oviposition, an infected palm dying in 2–4 months.

MAJOR SPECIES: this is a large genus with many described species, although only two, *B. cocophilus* and *B. xylophilus*, are currently considered to be of major economic importance.

DISTRIBUTION: *B. cocophilus* is restricted to the Caribbean, Central and South American regions, while *B. xylophilus* is recorded from some tropical/subtropical regions, including Hong Kong and southern China, but mainly occurs in more temperate climates, e.g. Japan and Portugal.

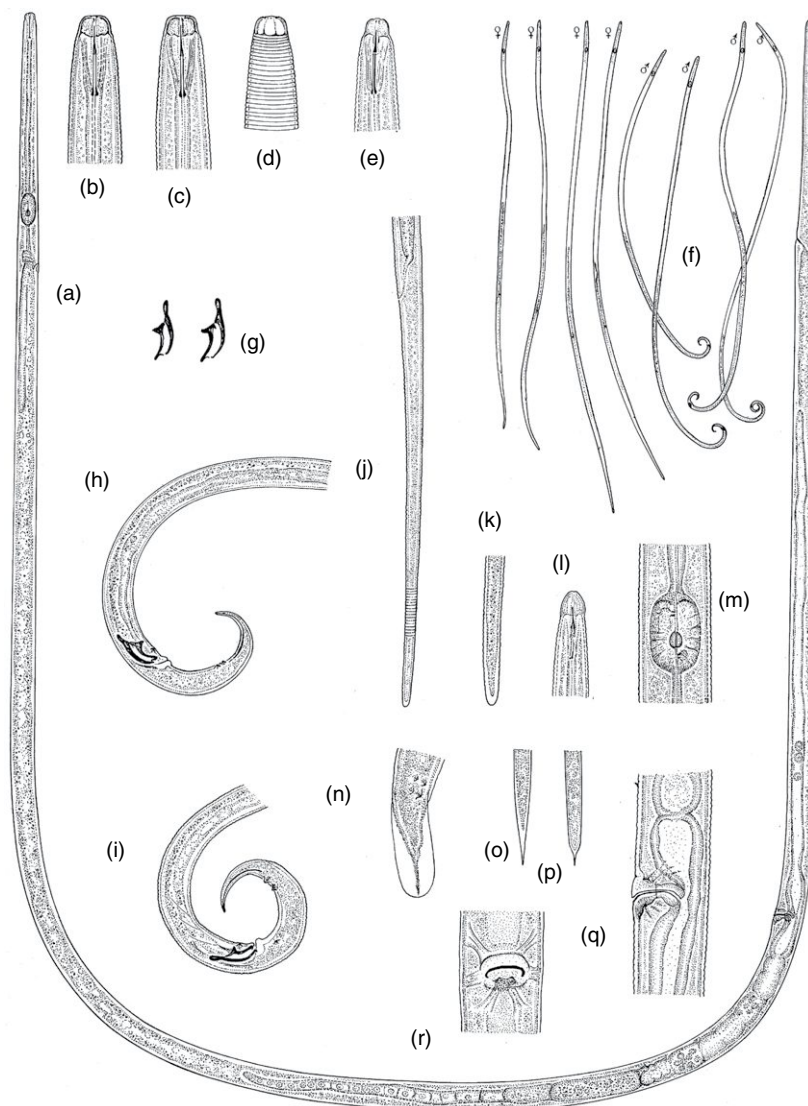


Fig. 2.4. *Bursaphelenchus cocophilus* (a) entire female; (b–d) female labial region; (e) male labial region; (f) entire females and males; (g) spicules; (h and i) male tail end; (j) female tail; (k) female tail tip; (l) juvenile labial region; (m) median bulb; (n) male 'bursal' flap; (o and p) juvenile tail tips; (q) vulval region; (r) vulval slit in ventral view. Line drawings are for illustrative purposes only and are not to scale.

Useful literature

- Braasch, H. and Schönfeld, U. (2015) Improved morphological key to the species of the *xylophilus* group of the genus *Bursaphelenchus* Fuchs, 1937. *Bulletin OEPP/EPPO Bulletin* 45, 73–80.
- Burgermeister, W., Braasch, H., Metge, K., Gu, J., Schröder, T. and Woldt, E. (2009) ITS-RFLP analysis, an efficient tool for differentiation of *Bursaphelenchus* species. *Nematology* 11, 649–668.
- Hunt, D.J. (1993) *Aphelenchida, Longidoridae and Trichodoridae: Their Systematics and Bionomics*. CAB International, Wallingford, UK.
- Hunt, D.J. (2008) A checklist of the Aphelenchoidea (Nematoda: Tylenchina). *Journal of Nematode Morphology and Systematics* 10, 99–135.

Kanzaki, N. (2008) Taxonomy and systematics of the nematode genus *Bursaphelenchus* (Nematoda: Parasitaphelenchidae). In: Zhao, B.G., Futai, K., Sutherland, J.R. and Takeuchi (eds) *Pine Wilt Disease*. Springer, Tokyo, pp. 44–66.

Kanzaki, N. and Giblin-Davis, R. (2012) Aphelenchoidea. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 161–208.

***Ditylenchus* Filipjev, 1936 (Tylenchina, Anguinidae) (Fig. 2.5)**

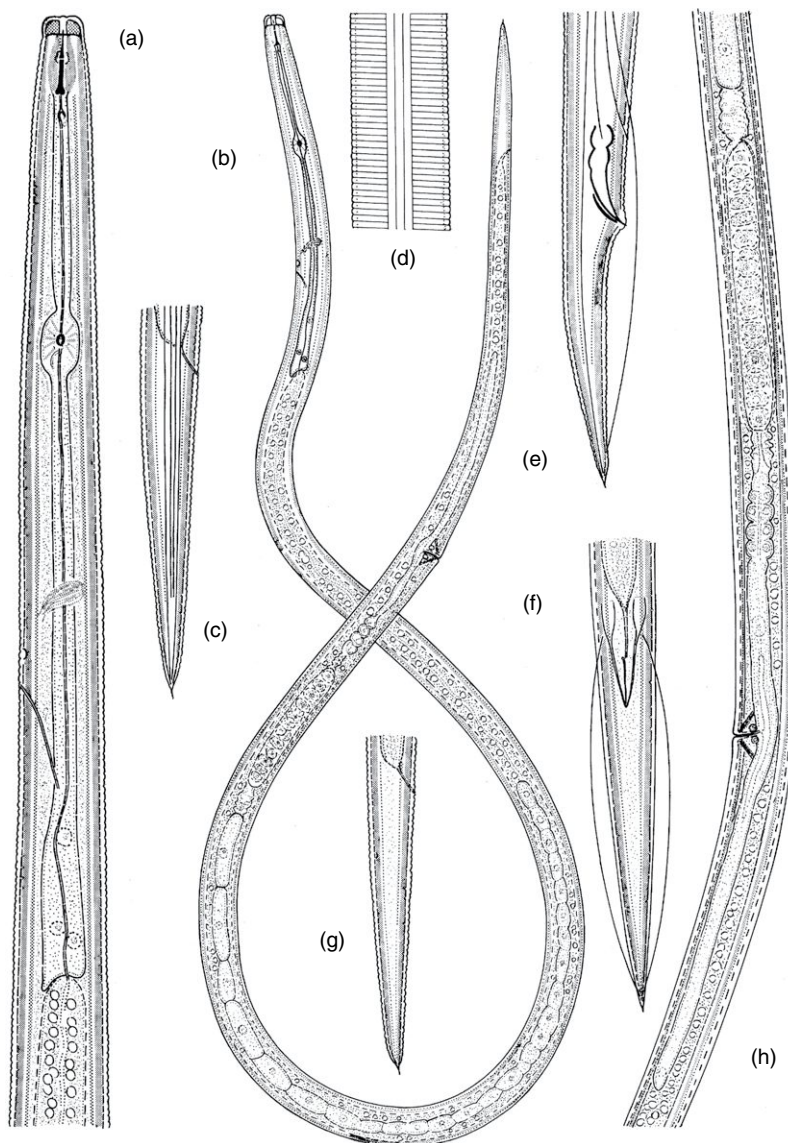


Fig. 2.5. *Ditylenchus angustus* (a) female pharyngeal region; (b) entire female; (c and g) female tails; (d) lateral field; (e and f) male tails; (h) female genital tract. Line drawings are for illustrative purposes only and are not to scale.

MORPHOLOGY: **slender nematodes**, dying straight or slightly curved ventrally on heat relaxation. Skeleton of labial region weakly sclerotized. Stylet of moderate strength and with small basal knobs. Pharynx with a muscular median bulb; **isthmus gradually expanding to form the basal bulb**, which may extend as a lobe over the intestine. Female: **vulva located well posterior. Genital tract single, anteriorly outstretched. Post-uterine sac present. Tail elongate, conoid.** Male: **bursa adanal, not reaching tail tip. Tail elongate, conoid.**

BIOLOGY: ectoparasites of plant stems and leaves but also found within the tissues. Infected stems and leaves are often stunted and deformed.

MAJOR SPECIES: a large genus, most species of which are fungal feeders. Major phytoparasitic species include *D. africanus*, *D. angustus*, *D. arachis*, *D. dipsaci*, *D. destructor* and *D. gigas*.

DISTRIBUTION: *D. angustus* is found in rice-growing areas of Bangladesh, Vietnam and other areas of Asia; *D. dipsaci* is restricted to the cooler regions of the tropics and subtropics, *D. africanus* is known only from South Africa and Mozambique, while *D. gigas* (formerly known as the giant race of *D. dipsaci*) is known from Europe, Africa and Asia. *D. arachis* was described from groundnuts in China.

CONFUSABLE GENERA: *Aphelenchoides*, *Anguina* (juvenile stages).

Useful literature

Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 225–269.

Subbotin, S.A. and Riley, I.T. (2012) Stem and gall forming nematodes. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 521–577.

***Anguina* Scopoli, 1777 (Tylenchina, Anguinidae) (Fig. 2.6)**

MORPHOLOGY: **sexually dimorphic. Adult stages only found in plant galls, juveniles occurring in galls, plant tissue or soil**, depending on the life cycle stage. General morphology similar to that of *Ditylenchus*. Female: obese, medium to large nematodes (1.5–5 mm), **dying spirally coiled** on heat relaxation. **Vulva very posterior with a single, anteriorly directed genital tract reflexed twice or more.** Numerous oocytes. Male: small to medium sized (1–2.5 mm), dying ventrally or dorsally (e.g. as in *A. tritici*) arcuate.

Testis well developed with one or more flexures. Bursa adanal.

BIOLOGY: forming galls on stems, leaves or flowers of various plants. The J2 stage is found in the soil and feeds ectoparasitically on the plant tissues. The final moult takes place after gall formation, each female laying up to 2000 eggs. As the gall matures and dries, the J2 infectives slowly desiccate to an anhydrobiotic state and may survive many years.

MAJOR SPECIES: *A. agrostis* complex, *A. tritici*.

CONFUSABLE GENUS: *Ditylenchus*, as the soil-dwelling juveniles look similar.

Useful literature

Subbotin, S.A. and Riley, I.T. (2012) Stem and gall forming nematodes. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 521–577.

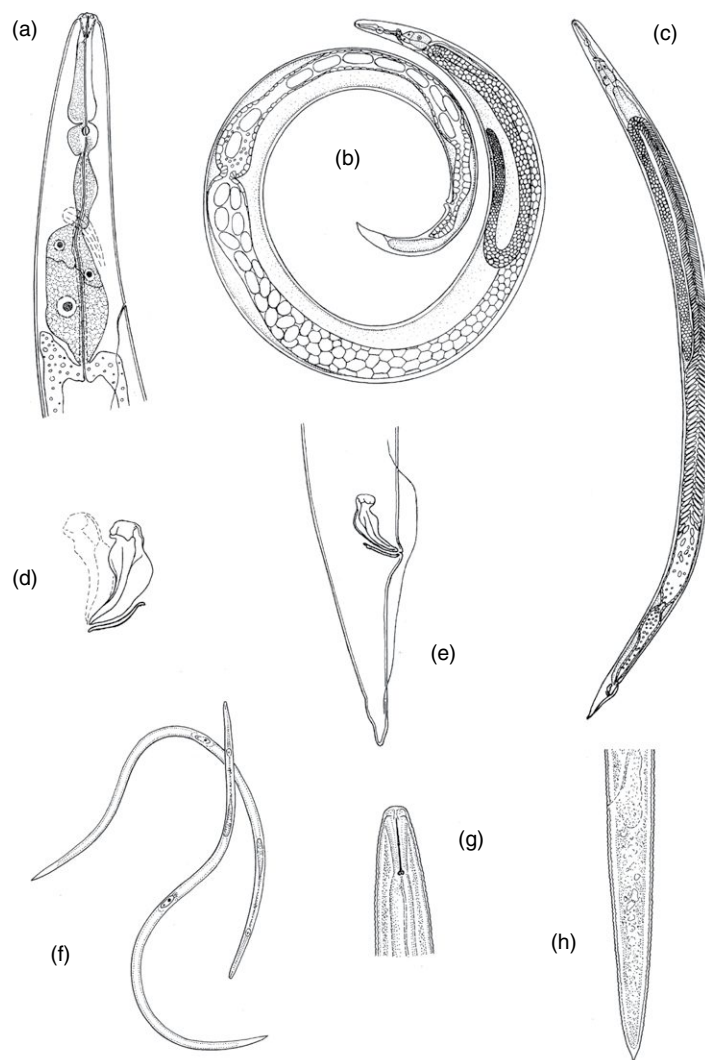


Fig. 2.6. *Anguina tritici* (a) female pharynx; (b) entire female; (c) entire male; (d) male spicules; (e) male tail; (f) second-stage juveniles; (g) J2 labial region; (h) J2 tail. (From Goodey, 1932; Thorne, 1949; Siddiqi, 1972, in CIH Descriptions Set, CAB International.) Line drawings are for illustrative purposes only and are not to scale.

***Tylenchorhynchus* Cobb, 1913 (Tylenchina, Belonolaimidae) = *Telotylenchus*, *Trilineellus*, *Divittus*, *Morasinema*, *Tessellus*, *Mulkorhynchus* (Fig. 2.7)**

MORPHOLOGY: small nematodes (rarely over 1 mm long), dying more or less straight or slightly curved ventrally on application of gentle heat. **No marked**

sexual dimorphism in form of anterior region. Labial region rounded, continuous with body contour or slightly offset, with narrow annuli and weak sclerotization. **Stylet slender, 15–30 μ m long, moderately sclerotized with rounded, backwardly sloping, knobs.** Lateral field with two, three or four lines; cuticle sometimes divided into blocks. Pharynx equally developed

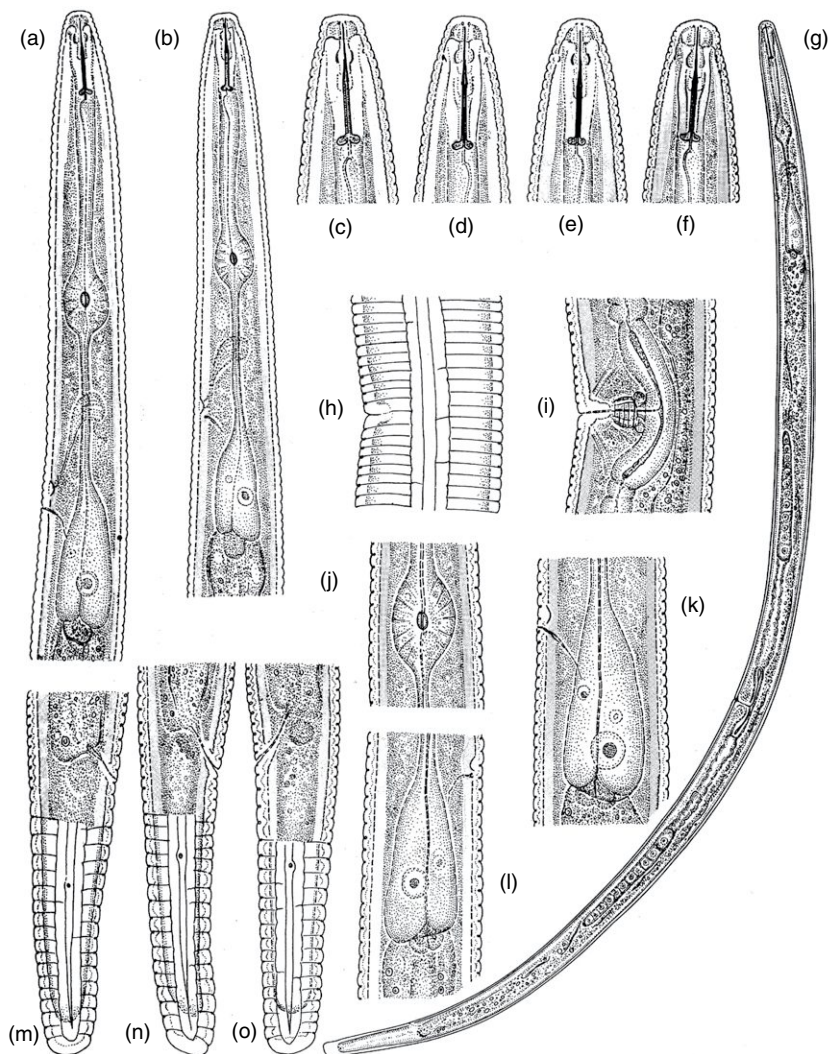


Fig. 2.7. *Tylenchorhynchus annulatus* (a and b) pharynx; (c–f) labial regions; (g) entire female; (h) lateral field; (i) vulval region; (j) median pharyngeal bulb; (k and l) basal pharyngeal bulb; (m–o) female tails. (From Siddiqi, 1976, in CIH Descriptions Set, CAB International.) Line drawings are for illustrative purposes only and are not to scale.

in both sexes; median bulb fusiform, moderately developed; pharyngeal glands abutting the intestine or, very rarely, overlapping. Female: **vulva median with two equally developed genital tracts**; one directed anteriorly, one posteriorly. Spermatheca rounded. **Tail, about three anal body diameters long, conoid to subcylindrical, with rounded tip.** Male: tail elongate,

conical-pointed, bursa extending to tail tip, trilobed in some species. Spicules slightly curved.

BIOLOGY: migratory ecto-, semi-ecto- or endoparasites. Most species are bisexual. Polyphagous. Not considered as being very important parasites. Well distributed in all climatic areas.

MAJOR SPECIES: *T. annulatus*, *T. brassicae*, *T. mashhoodi*.

CONFUSABLE GENERA: *Dolichorhynchus*, *Neodolichorhynchus*, *Trichotylenchus*, *Quinisulcius*, *Merlinius*, *Amplimerlinius*.

Useful literature

- Geraert, E. (2011) *The Dolichodoridae of the World – Identification of the Family Dolichodoridae (Nematoda)*. Academia Press, Ghent, Belgium, 430 pp.
- Handoo, Z.A. (2000) A key and diagnostic compendium to the species of the genus *Tylenchorhynchus* Cobb, 1913 (Nematoda: Belonolaimidae). *Journal of Nematology* 32, 20–34.
- Handoo, Z.A., Palomares-Rius, J.E., Cantalapiedra-Navarrete, C., Liébanas, G., Subbotin, S.A. and Castillo, P. (2014) Integrative taxonomy of the stunt nematodes of the genera *Bitylenchus* and *Tylenchorhynchus* (Nematoda, Telotylenchidae) with description of two new species and a molecular phylogeny. *Zoological Journal of the Linnean Society* 172, 231–264.
- Hunt, D.J., Bert, W. and Siddiqi, M.R. (2012) Tylenchidae and Dolichodoridae. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 209–250.
- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 429–508.

***Pratylenchus* Filipjev, 1936 (Tylenchina, Pratylenchidae) (Fig. 2.8)**

MORPHOLOGY: small nematodes (<1 mm long), dying slightly curved ventrally on application of gentle heat. No marked sexual dimorphism in form of anterior region. **Labial region strongly sclerotized, low, flattened**, usually appearing as a dark, flat cap under the stereomicroscope, **divided into two, three or four annuli and continuous with the body contour. Stylet is approximately 20 µm or less in length** (i.e. less than three times as long as the labial region diameter), moderately sclerotized and with rounded or anteriorly concave knobs. Pharynx equally developed in both sexes, median bulb well developed; **pharyngeal gland lobes overlapping intestine ventrally. Female: vulva well posterior at 70–80% of body length; genital system with a single, anteriorly directed tract** (monoprodelfic) and a variable post-vulval section, which may show some differentiation, but which is never functional; spermatheca oval or round and usually filled with sperm in bisexual species. **Tail subcylindroid or more or less conoid, with a broad to narrowly rounded or truncate terminus**, which may be smooth or annulated. Male: tail short, dorsally convex-conoid; **bursa extending to tail tip**; spicules slender, arcuate.

BIOLOGY: migratory endoparasites with all stages found in the root cortex. Low soil populations can be associated with high root populations. The nematodes feed mainly on cortex cells and form cavities containing 'nests' or colonies of nematodes of all stages. Discoloration of affected tissues is usually pronounced. Above-ground symptoms of attack include chlorosis and stunting. Some species reproduce sexually, while others are parthenogenetic. The life cycle may be completed in 3–4 weeks and the nematodes can survive in the absence of host plants for several months. Most important species are polyphagous, although *Pratylenchus goodeyi* may be restricted to banana. Cryptic speciation appears to be widespread in the genus.

MAJOR SPECIES: *P. brachyurus*, *P. coffeae*, *P. goodeyi*, *P. penetrans*, *P. vulnus*, *P. zeae*.

DISTRIBUTION: *P. brachyurus*, *P. coffeae* and *P. zeae* are widely distributed in tropical and subtropical areas; *P. penetrans* mainly in cooler regions of the tropics; *P. goodeyi* on banana in Crete and the Canary Islands and in the cooler areas of Ethiopia, Kenya, Tanzania, Uganda and Burundi.

CONFUSABLE GENUS: *Radopholus*. Novices may confuse with *Aphelenchus avenae*, particularly in populations of the latter where there are abundant males.

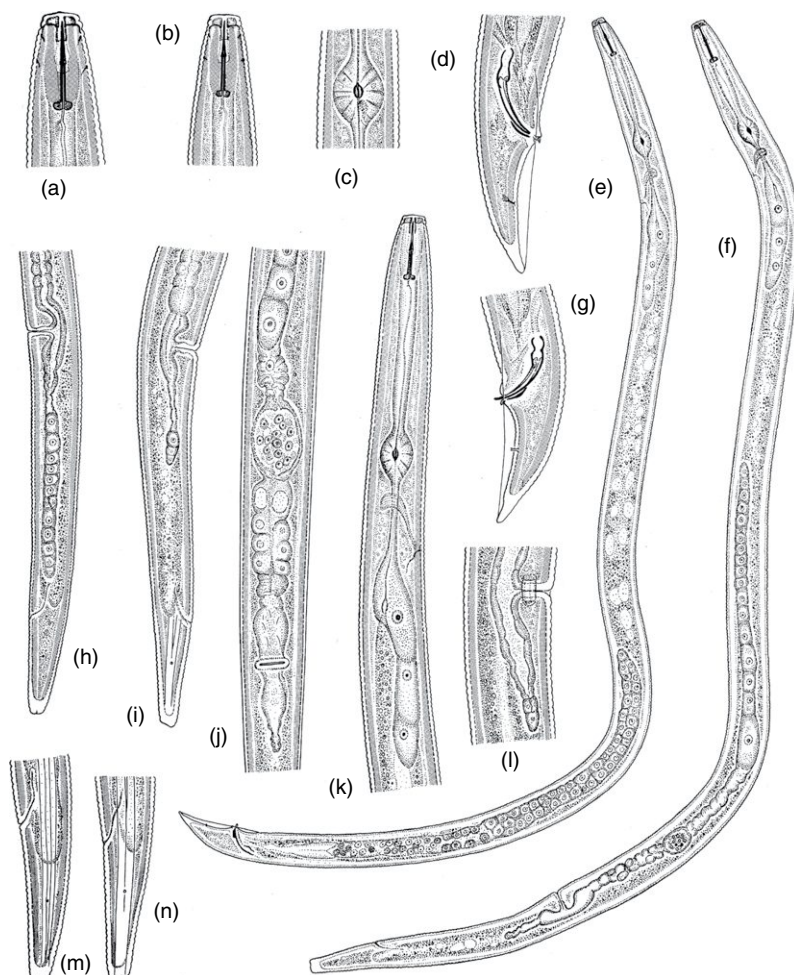


Fig. 2.8. *Pratylenchus coffeae* (a) female labial region; (b) male labial region; (c) median bulb; (d and g) male tail; (e) entire male; (f) entire female; (h and i) female posterior region; (j) female vulval region, ventral view; (k) pharyngeal region; (l) vulval region; (m and n) female tails. (From Siddiqi, 1976, in CIH Descriptions Set, CAB International.) Line drawings are for illustrative purposes only and are not to scale.

Useful literature

- Castillo, P. and Vovlas, N. (2007) *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. In: Hunt, D.J. and Perry, R.N. (series eds) *Nematology Monographs and Perspectives* 6. Brill, Leiden, the Netherlands, 529 pp.
- Castillo, P., Stanley, J., Inserra, R.N. and Manzanilla-López, R.H. (2012) Pratylenchidae – the lesion nematodes. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 411–478.
- Geraert, E. (2013) *The Pratylenchidae of the World – Identification of the Family Pratylenchidae* (Nematoda). Academia Press, Ghent, Belgium, 430 pp.
- Palomares-Rius, J.E., Castillo, P., Liébanas, G., Vovlas, N., Landa, B.B., *et al.* (2010) Description of *Pratylenchus hispaniensis* n. sp. from Spain and considerations on the phylogenetic relationship among selected genera in the family Pratylenchidae. *Nematology* 12, 429–451.
- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 336–366.

***Hirschmanniella* Luc & Goodey,
1963 (Tylenchina, Pratylenchidae)**
(Fig. 2.9)

MORPHOLOGY: medium sized to long, slender nematodes (1.1–4 mm), **dying more or less straight or ventrally arcuate** on application of gentle heat. No marked sexual dimorphism in form of anterior region. **Labial region**

continuous with body contour, **hemispherical or anteriorly flattened, annulated. Stylet strongly developed** (15–46 µm), with rounded basal knobs. **Pharyngeal glands elongate and overlapping intestine in a long ventral lobe.** Female: **vulva median; genital system with two functional and equally developed genital tracts, one anteriorly and one posteriorly directed; tail elongate, conoid,**

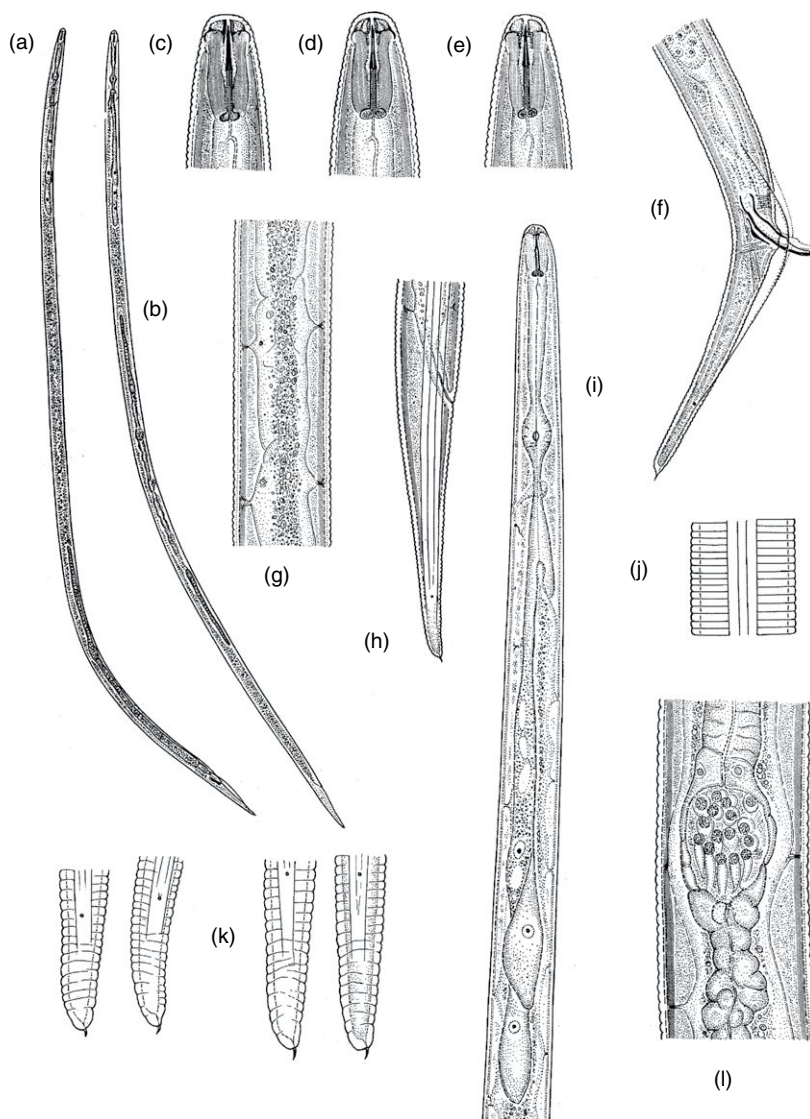


Fig. 2.9. *Hirschmanniella oryzae* (a) entire male; (b) entire female; (c–e) labial region; (f) male tail; (g) mid-body showing 'Thorneian cells'; (h) female tail; (i) pharyngeal region; (j) lateral field; (k) female tail tips; (l) spermatheca with sperm. (From Siddiqi, 1973, in CIH Descriptions Set, CAB International.) Line drawings are for illustrative purposes only and are not to scale.

terminal mucron often present. Male tail similar to female; bursa not reaching to tail tip, spicules slender, arcuate.

BIOLOGY: migratory endoparasites, mainly of roots, but also corms and rhizomes, where they move freely through the tissues. Eggs are laid within the root, and development to the adult takes about 5–6 weeks. The genus is associated with aquatic environments – marsh, freshwater and marine. Most species are bisexual.

MAJOR SPECIES: *H. mexicana* (= *caudacrena*), *H. imamuri*, *H. miticausa*, *H. mucronata*, *H. oryzae*, *H. spinicaudata*.

DISTRIBUTION: the genus is distributed worldwide in suitable habitats, with *H. oryzae*, the major species, being widely distributed in the rice-growing areas of India, Bangladesh, Malaysia, Indonesia, the Philippines and Japan. It is also found in parts of Africa and South America.

CONFUSABLE GENUS: *Radopholus*.

Useful literature

- Castillo, P., Stanley, J., Inserra, R.N. and Manzanilla-López, R.H. (2012) Pratylenchidae – the lesion nematodes. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 411–478.
- Geraert, E. (2013) *The Pratylenchidae of the World – Identification of the Family Pratylenchidae (Nematoda)*. Academia Press, Ghent, Belgium, 430 pp.
- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 336–366.

***Radopholus* Thorne, 1949 (Tylenchina, Pratylenchidae) = *Neoradopholus* (Fig. 2.10)**

MORPHOLOGY: small nematodes (<1 mm long), dying more or less straight or slightly curved ventrally when heat relaxed. **Marked sexual dimorphism in form of anterior region: female labial region low, rounded, continuous or slightly offset from body contour; male labial region higher, often knob-like and more offset. Male labial sclerotization, stylet and pharynx reduced;** female cephalic sclerotization strong, stylet and pharynx well developed. Median bulb in female pharynx well developed and **pharyngeal glands mostly overlapping intestine dorsally**. Female: **vulva median, with two functional and equally developed genital tracts**, spermathecae rounded and with sperm in bisexual species; tail elongate, conoid (c. 60 µm long in *Radopholus similis*). Male: tail elongate, conoid, ventrally arcuate; **bursa not reaching to tail tip in most species**, including *R. similis*; spicules slender, arcuate.

activities are restricted to the cortex, causing cavitation, discoloration and severe damage, allowing secondary invasion by other micro-organisms. The adult male is non-feeding. The major species is *R. similis*, which has two recognized host races or biotypes, one attacking banana and many other plants, but not citrus, the other (previously recognized as a separate species, *Radopholus citrophilus*, by some authorities) attacking both citrus and banana, as well as a variety of other plants. It is possible that *R. similis* includes a range of host races, current evidence also indicating a highly variable pathogenicity.

MAJOR SPECIES: *R. similis*, *R. citri*, *R. bridgei*, *R. duriophilus*, *R. musicola*.

DISTRIBUTION: the majority of species have been described from Australasia. However, *R. similis* has been introduced worldwide in tropical regions and occurs virtually everywhere that banana is grown. The citrus race of *R. similis* is only recorded from Florida.

BIOLOGY: migratory endoparasites of root and corm/tuber tissues. In roots, the feeding

CONFUSABLE GENERA: *Achlysiella*, *Pratylenchus*, *Hirschmanniella*.

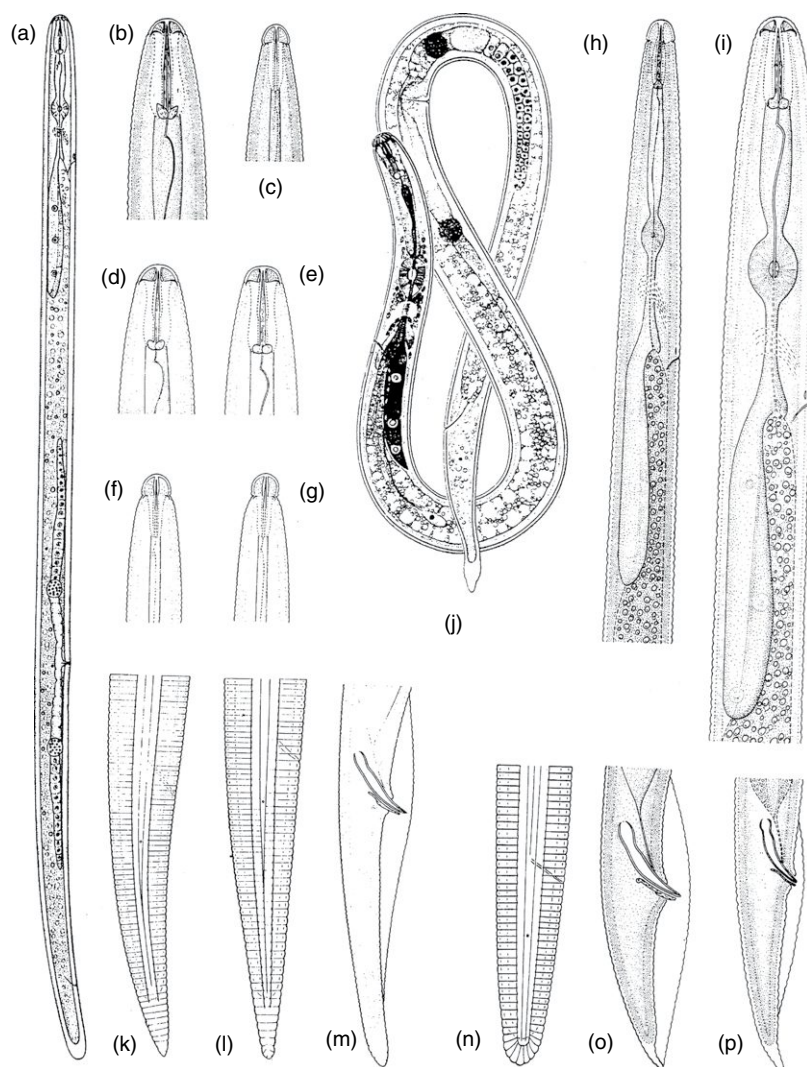


Fig. 2.10. (a) *Radopholus rotundisemenus*, (h, i and p) *Radopholus vangundyi*, (b, c, n and o) *Radopholus inaequalis* and (d–g and j–m) *Radopholus similis*. (a) Entire female; (b, d and e) female labial region; (c, f and g) male labial region; (h) male pharynx; (i) female pharynx; (j) entire female; (k and l) female tails; (m) male tail; (n) female tail; (o) male tail; (p) male tail. Line drawings are for illustrative purposes only and are not to scale.

Useful literature

- Castillo, P., Stanley, J., Inserra, R.N. and Manzanilla-López, R.H. (2012) Pratylenchidae – the lesion nematodes. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 411–478.
- Geraert, E. (2013) *The Pratylenchidae of the World – Identification of the Family Pratylenchidae (Nematoda)*. Academia Press, Ghent, Belgium, 430 pp.
- Ryss, A.Y. (2003) Taxonomy, evolution and phylogeny of the genus *Radopholus* (didelphic species) according to morphological data, with a key to species (Nematoda: Tylenchida). *Zoosystematica Rossica* 11, 243–256.
- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 336–366.

***Nacobbus* Thorne & Allen, 1944**
(Tylenchina, Pratylenchidae) (Fig. 2.11)

MORPHOLOGY: **sexually dimorphic.** Immature female (in soil or in roots). Vermiform, slender, 0.6–1 mm long. Labial area rounded, continuous with body contour. Labial sclerotization strong; stylet robust, with rounded basal knobs.

Pharynx with strong median bulb and strong valves; **pharyngeal glands long, overlapping intestine dorsally.** **Vulva located posteriorly** ($V = 90\text{--}95\%$); vulval lips not protruding. **Single anterior genital tract present.** **Tail short, rounded.** **Mature females (in roots): body saccate; anterior and posterior portions tapering.** Genital tract convoluted. Tail short.

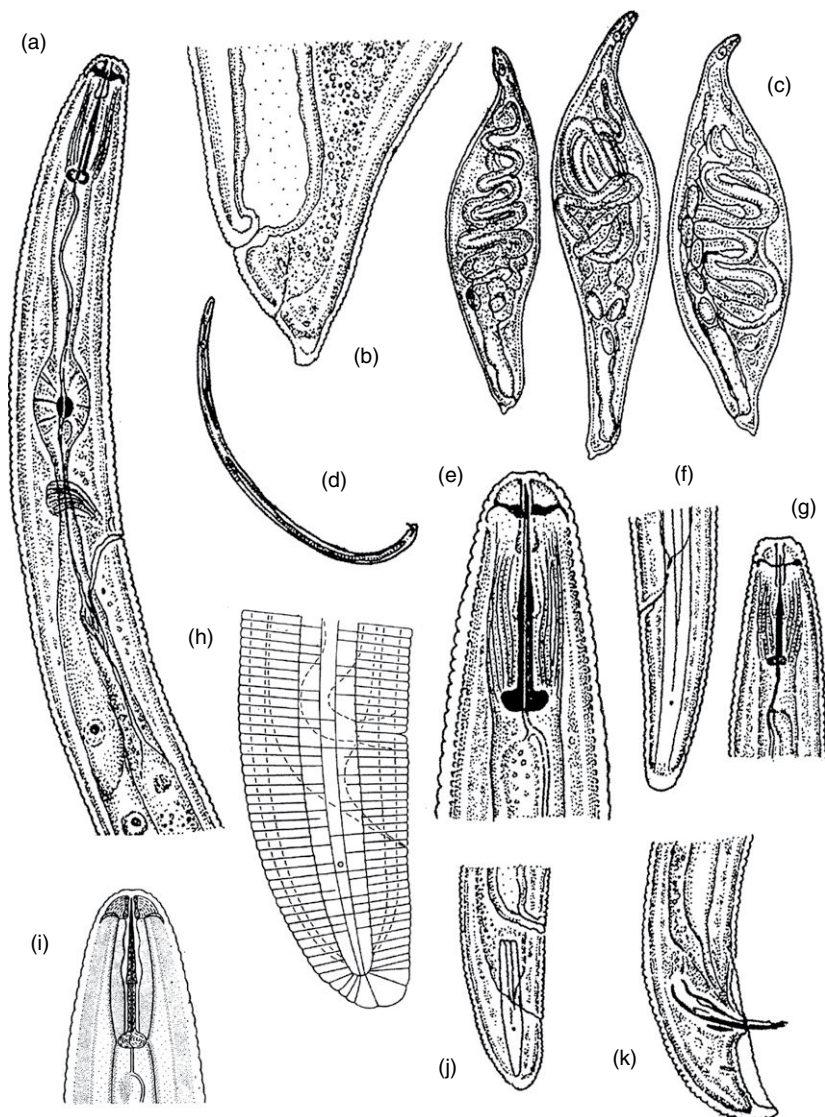


Fig. 2.11. *Nacobbus aberrans* (a) male pharyngeal region; (b) tail region of mature female; (c) mature females; (d) entire male; (e and i) male labial region; (f) tail region of second-stage juvenile; (g) labial region of second-stage juvenile; (h and j) immature female posterior region; (k) male tail. Line drawings are for illustrative purposes only and are not to scale.

Male: similar to immature female, except for sexual characters. Spicules curved. Tail short; bursa reaching tail tip. Juveniles: uncoiled J4 resembles immature female.

BIOLOGY: in some species, the eggs are laid within a gelatinous matrix formed by the female. On hatching, the J2 invades a root, but does not form a fixed feeding site. Instead, the juveniles migrate through the tissue and may even leave the root and enter another. The J3 and J4 stages are less mobile. After the final moult, the immature female may leave the root and enter another before taking up position near the vascular tissue and initiating a syncytial trophic system and gall formation. As the female develops, the posterior region extends towards the epidermis

and an opening in the gall is formed through which the gelatinous matrix and eggs are extruded. In another species, *Nacobbus dorsalis*, the eggs are retained within the female body.

MAJOR SPECIES: *N. aberrans*, *N. bolivianus*, *N. dorsalis*.

DISTRIBUTION: indigenous to the Americas and only known to be established there.

CONFUSABLE GENUS: mature females may be confused with *Meloidogyne*. Under the stereomicroscope, immature vermiform females may be confused with *Meloidogyne* males, and the coiled J3 or J4 juveniles may be confused with *Helicotylenchus*.

Useful literature

- Castillo, P., Stanley, J., Inserra, R.N. and Manzanilla-López, R.H. (2012) Pratylenchidae – the lesion nematodes. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 411–478.
- Lax, P., Rondan Dueñas, J.C., Gardenal, C.N. and Doucet, M.E. (2013) Phylogenetic relationships among populations of the *Nacobbus aberrans* (Nematoda, Pratylenchidae) complex reveal the existence of cryptic species. *Zoologica Scripta* 43, 184–192.
- Manzanilla-López, R.H. (2010) Speciation within *Nacobbus*: consilience or controversy? *Nematology* 12, 321–334.
- Manzanilla-López, R.H., Costilla, M.A., Doucet, M., Franco, J., Inserra, R.N., et al. (2003) The genus *Nacobbus* Thorne & Allen, 1944 (Nematoda: Pratylenchidae): systematics, distribution, biology and management. *Nematopica* 32, 149–227.
- Reid, A., Manzanilla-López, R.H. and Hunt, D.J. (2003) *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 (Nematoda: Pratylenchidae); a nascent species complex revealed by RFLP analysis and sequencing of the ITS-rDNA region. *Nematology* 5, 441–451.
- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 366–369.

Helicotylenchus Steiner, 1945 (Tylenchina, Hoplolaimidae) = *Rotylenchoides*, *Zimmermannia* (Fig. 2.12)

MORPHOLOGY: small to medium-sized nematodes (0.4–1.2 mm), **usually dying in a spiral** (rarely 'c'-shaped) on heat relaxation. **Labial region conoid-rounded, rarely truncate, sclerotization moderate. Stylet well developed, usually 3–4 times the lip region diameter in length and with rounded or cup-shaped knobs. Opening of dorsal pharyngeal gland duct 25–50% of stylet length posterior to knobs. Pharyngeal gland lobe overlapping intestine mainly ventrally.** Female: vulva posterior

(60–70%), **both genital tracts usually fully developed**, posterior branch rarely reduced and non-functional (= *Rotylenchoides*). **Tail short, usually dorsally convex-conoid or hemispherical.** A terminal projection or mucron may be present. **Phasmids small, dot-like.** Male: tail short, spicules well developed, arcuate. Bursa reaching tail tip.

BIOLOGY: ectoparasitic, semi-endoparasitic or endoparasitic nematodes of roots. All stages can be found in the root cortex, but migration through the tissues has not been reported. Small lesions are formed that become necrotic as secondary invasion proceeds. Polyphagous.

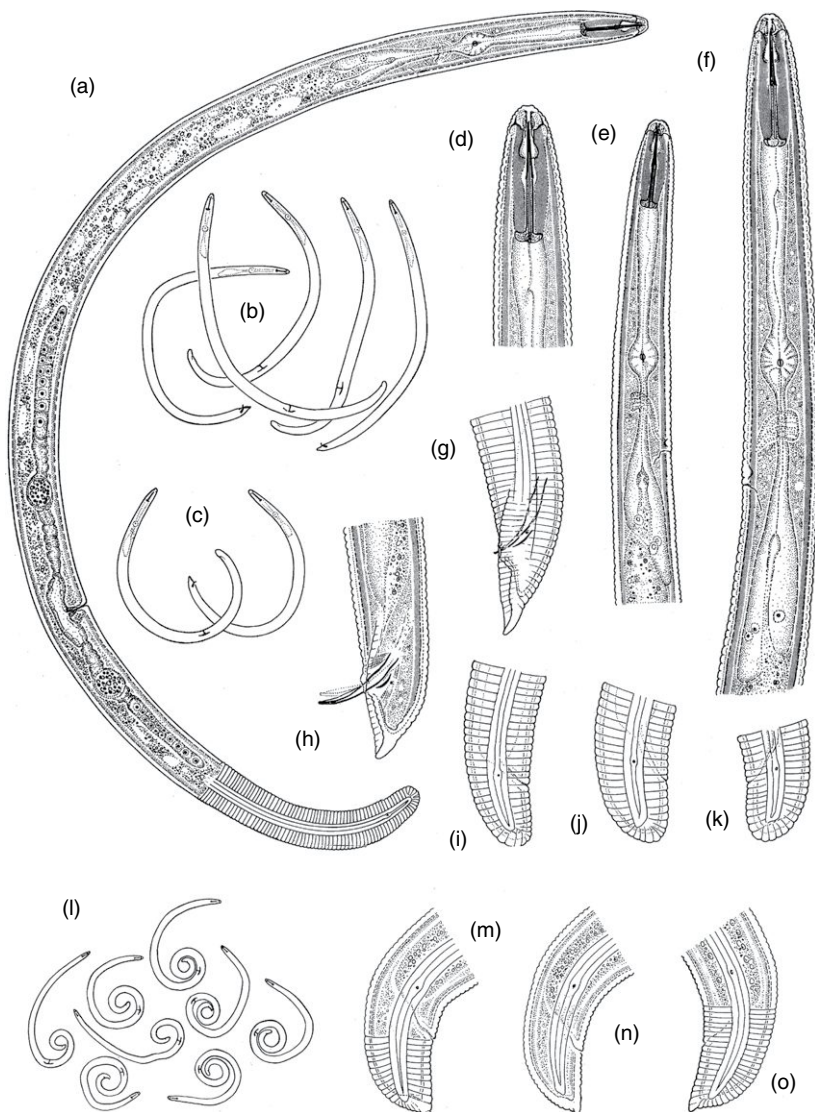


Fig. 2.12. *Helicotylenchus multicinctus* (a) entire female; (b) females; (c) males; (d) female labial region; (e) female pharynx; (f) male pharynx; (g and h) male tails; (i–k) female tails. *Helicotylenchus dihystra* (l) females; (m–o) female tails. (From Siddiqi, 1972, 1973, in CIH Descriptions Set, CAB International.) Line drawings are for illustrative purposes only and are not to scale.

Most species are parthenogenetic but one of the most common and most damaging species, *Helicotylenchus multicinctus*, is amphimictic. Cryptic species complexes are known to occur.

MAJOR SPECIES: *H. dihystra*, *H. erythrinae*, *H. mucronatus*, *H. multicinctus*, *H. pseudorobustus*.

DISTRIBUTION: throughout the tropical and sub-tropical areas.

CONFUSABLE GENUS: *Rotylenchus* (has the dorsal pharyngeal gland duct opening more anterior and dorsally overlapping gland lobe). J2 stage may be confused with *Rotylenchulus* juveniles.

Useful literature

- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 301–308.
- Subbotin, S.A., Vovlas, N., Yeates, G.W., Hallmann, J., Kiewnick, S., *et al.* (2015) Morphological and molecular characterisation of *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 and related species (Tylenchida: Hoplolaimidae) with a phylogeny of the genus. *Nematology* 17, 27–52.
- Van den Berg, E. and Quénéhervé, P. (2012) Hoplolaimidae. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 251–297.

***Hoplolaimus* von Daday, 1905**
(Tylenchina, Hoplolaimidae) = *Basirolaimus*,
***Hoplolaimoides* (Fig. 2.13a–g)**

MORPHOLOGY: nematodes of medium length (1–2 mm), dying slightly curved ventrally on application of gentle heat. **Labial region high, offset**, rounded and **with massive sclerotization**. Basal lip annulus may be divided into small squares. **Stylet massive, 40–50 µm long, with well-developed basal knobs bearing anterior tooth-like projections**. Pharynx well developed with a **dorsally overlapping gland lobe** containing either three or six (= *Basirolaimus*) nuclei. Female: vulva median, genital system consisting of two opposed tracts. Tail short, bluntly rounded. **Phasmids enlarged to form scutella, one being between anus and vulva and the other anterior to vulva**. Male: tail short, spicules well developed, arcuate. Bursa extending to tail tip. **Scutella situated at similar relative positions to female**.

MAJOR SPECIES: *Hoplolaimus columbus*, *Hoplolaimus indicus*, *Hoplolaimus pararobustus*, *Hoplolaimus seinhorsti*.

***Scutellonema* Andrásy, 1958**
(Tylenchina, Hoplolaimidae)
(Fig. 2.13h–j)

MORPHOLOGY: small to medium-sized nematodes (0.3–1.5 mm), usually dying in a C-shape or open spiral. **Labial region with moderate sclerotization**. **Stylet of medium development with rounded knobs**. **Pharynx with**

dorsal overlap. Female: vulva median with two opposed genital tracts. Tail short, bluntly rounded. **Phasmids enlarged to form scutella, which are opposite one another and either on, or very near, the tail**. Male: tail short, spicules well developed, arcuate. Bursa extending to tail tip.

MAJOR SPECIES: *S. brachyurus*, *S. bradys*, *S. cavenessi*.

***Aorolaimus* Sher, 1964 (Tylenchina,**
Hoplolaimidae) = *Peltamigratus*,
***Nectopelta* (Fig. 2.13k–m)**

MORPHOLOGY: similar to *Scutellonema* in general characters but female differs in having **scutella located well anterior to anus (yet posterior to vulva) and not opposite one another**. Males have scutella similarly arranged to the female and a large bursa, which in many species is extended beyond the tail tip as two lobes.

MAJOR SPECIES: *Aorolaimus luci*.

BIOLOGY: *Hoplolaimus*, *Scutellonema* and *Aorolaimus* are migratory endoparasites of roots and/or tubers. Most species are polyphagous. Reproduction can be amphimictic or parthenogenetic. *S. bradys* causes a serious dry rot of yam tubers.

DISTRIBUTION: widespread in tropical and subtropical areas, although *Aorolaimus* is more restricted to South America and parts of Africa.

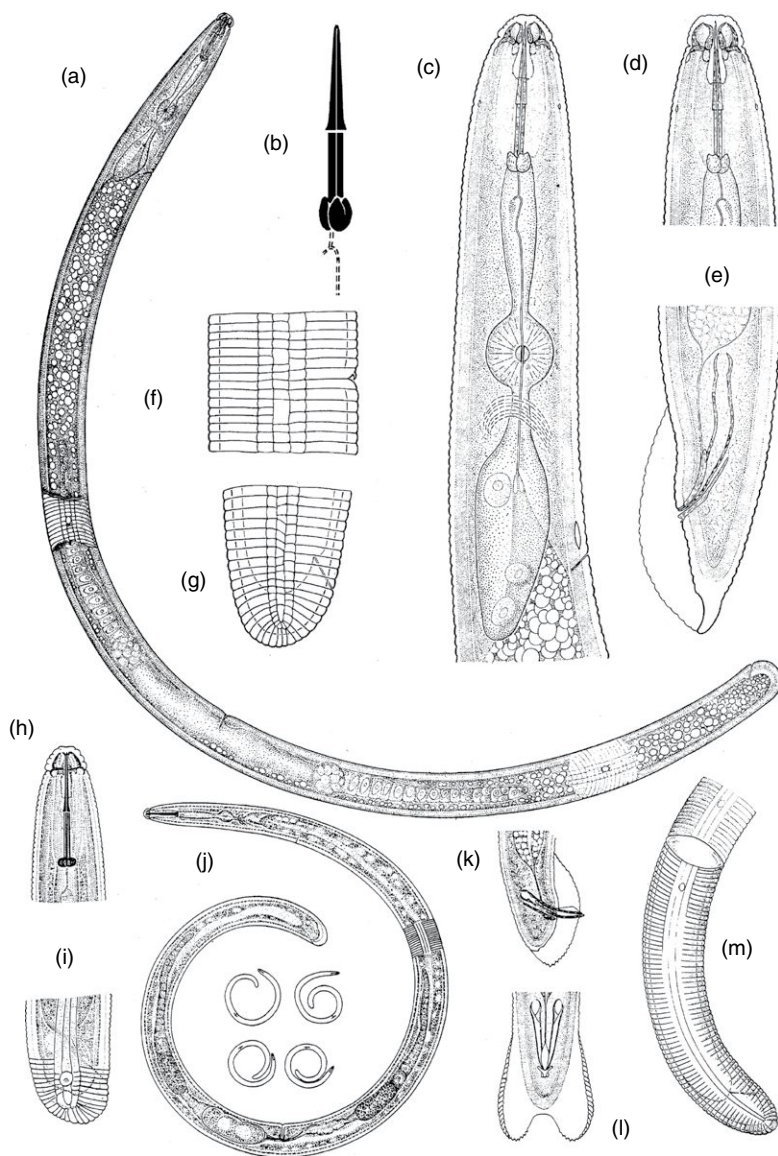


Fig. 2.13. *Hoplolaimus galeatus* (a) entire female; (c) female pharynx; (d) male labial region; (e) male tail; (f) vulval region and lateral field; (g) female tail. *Hoplolaimus seinhorsti* (b) stylet and tulip-shaped knobs. *Scutellonema brachyurus* (h) labial region; (i) female tail; (j) adult females. *Aorolaimus luci* (k) male tail, lateral view; (l) male tail, ventral view; (m) female posterior region showing scutella. Line drawings are for illustrative purposes only and are not to scale.

Useful literature

- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 278–297.
- Van den Berg, E. and Quénéhervé, P. (2012) Hoplolaimidae. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 251–297.

***Aphasmatylenchus* Sher, 1965 (Tylenchina, Hoplolaimidae) (Fig. 2.14)**

MORPHOLOGY: medium-sized nematodes (0.9–1.8 mm) assuming an open C-shape on heat relaxation. Weak sexual dimorphism in form of anterior region. **Annuli** prominent, either smooth or, as in *Aphasmatylenchus straturatus*, with **numerous longitudinal striae dividing each**

annulus into small blocks (maize-cob-like configuration). Labial region offset from body contour, annulated, conoid with distinct labial disc. **Stylet strongly developed, less than three labial region diameters long** and with rounded basal knobs. **Pharyngeal glands overlapping intestine in a mostly ventral lobe**. Intestinal fasciculi present, extending beyond rectum into tail. **Female: vulva median;**

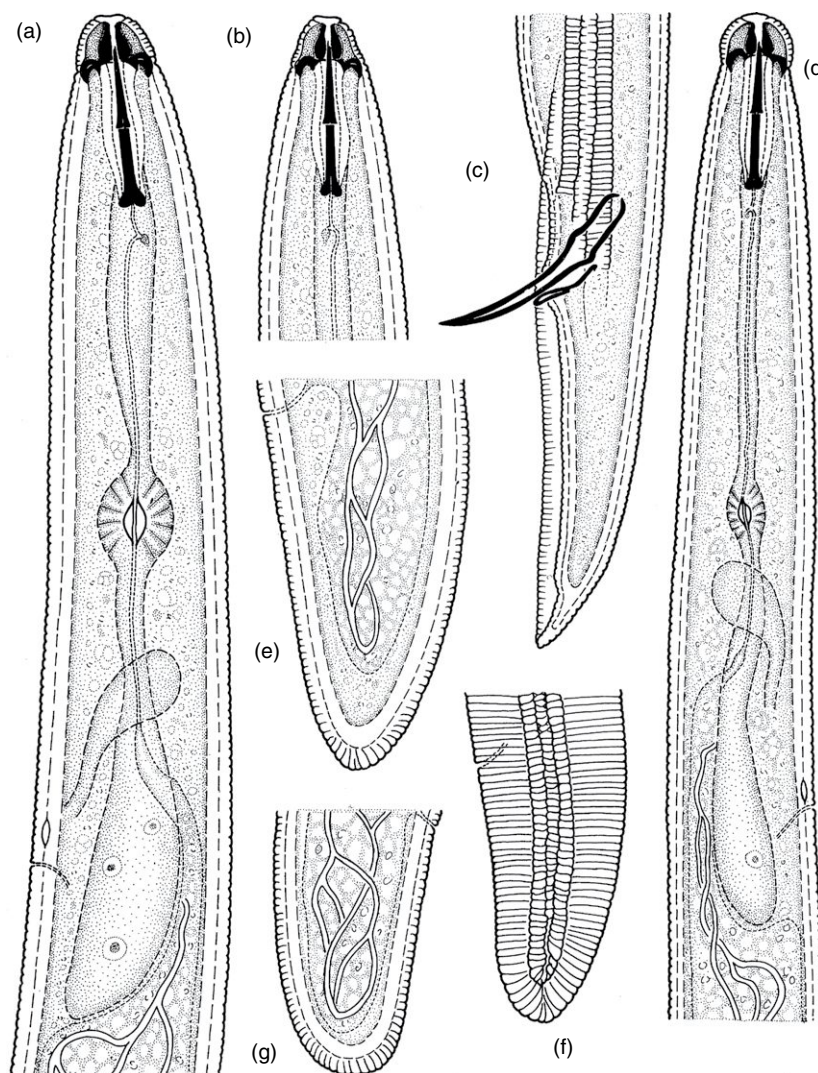


Fig. 2.14. *Aphasmatylenchus straturatus* (a) female anterior region; (b) female labial region; (c) male tail; (d) male anterior region; (e–g) female tail region showing intestinal fasciculi; (f) female tail region, surface view. (From Germani, 1977, in CIH Descriptions Set, CAB International.) Line drawings are for illustrative purposes only and are not to scale.

genital system with two functional and equally developed genital tracts, one anteriorly and one posteriorly directed; **tail cylindrical to conoid-rounded, phasmids absent. Male stylet and pharynx less well developed than in female, tail elongate conoid**, tapering to a pointed terminus; bursa reaching to tail tip. **Phasmids absent.** Spicules robust, arcuate.

BIOLOGY: usually migratory ectoparasites, although they may also be found inside roots. *A. straturatus* parasitizes legumes, including groundnut (where it causes 'voltaic chlorosis'), soybean, pigeonpea and cowpea. This species has also been associated with the shea butter tree (*Butyrospermum parkii*) throughout Burkina Faso. The nematodes do not appear to be capable

of entering an anhydrobiotic state, but migrate deeper into the soil horizon during the dry season. The type species, *Aphasmatylenchus nigeriensis*, was found in the rhizosphere of *Theobroma cacao* and *Hevea brasiliensis*.

MAJOR SPECIES: *A. straturatus*, *A. nigeriensis*, *A. liberiensis*.

DISTRIBUTION: the genus is predominantly found in the sahelian zone of West Africa, where it has been recorded from Nigeria, Burkina Faso (formerly Upper Volta), Côte d'Ivoire, Mali, Liberia and Senegal. *A. nigeriensis* has also been recorded from tropical rainforest in French Guyana, South America.

CONFUSABLE GENUS: *Scutellonema*, *Aorolaimus*.

Useful literature

- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 320–322.
- Van den Berg, E. and Quénéhervé, P. (2012) Hoplolaimidae. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 251–297.

***Rotylenchulus* Linford & Oliveira, 1940 (Tylenchina, Hoplolaimidae) (Fig. 2.15)**

MORPHOLOGY: **sexually dimorphic.** Immature female (free in soil): body vermiform, small (0.23–0.64 mm), dying ventrally arcuate on application of gentle heat. **Labial region continuous with body contour**, rounded to conoid, striated. Labial sclerotization of medium development. **Stylet of medium strength, with rounded basal knobs.** Pharynx with well-developed median bulb and valves; **dorsal pharyngeal gland opening located well posterior to stylet base** (0.6–1.9 times stylet length); **pharyngeal glands well developed with a long lateral overlap.** Vulva posteriorly situated ($V = 58\text{--}72$); vulval lips not protuberant. **Two genital tracts**, each with a double flexure. Tail conoid, with rounded terminus. **Mature female (on roots): swollen to kidney-shaped body.** Anterior part irregular. Vulval lips protruding. Genital tracts convoluted. Male: vermiform. Labial sclerotization, stylet and pharynx reduced (median pharyngeal bulb weak, without valves)

but conspicuous. Spicules curved. Tail pointed. **Bursa not reaching tail tip.** Juvenile: resembling immature female, but shorter and lacking vulva and genital tracts.

BIOLOGY: the eggs are laid in a gelatinous matrix. On hatching, the juveniles moult to the immature female or male without feeding. The immature female is the invasive stage, but only the anterior section penetrates the root tissue, the posterior part remaining in the soil and becoming obese (i.e. a sedentary semi-endoparasite). About 50 eggs are deposited in a gelatinous matrix secreted by specialized vaginal cells.

MAJOR SPECIES: *R. borealis*, *R. parvus*, *R. reniformis*.

DISTRIBUTION: *R. reniformis* is almost ubiquitous in tropical and subtropical soils, although the other species appear to be more restricted in distribution.

CONFUSABLE GENUS: *Senegalonema*.

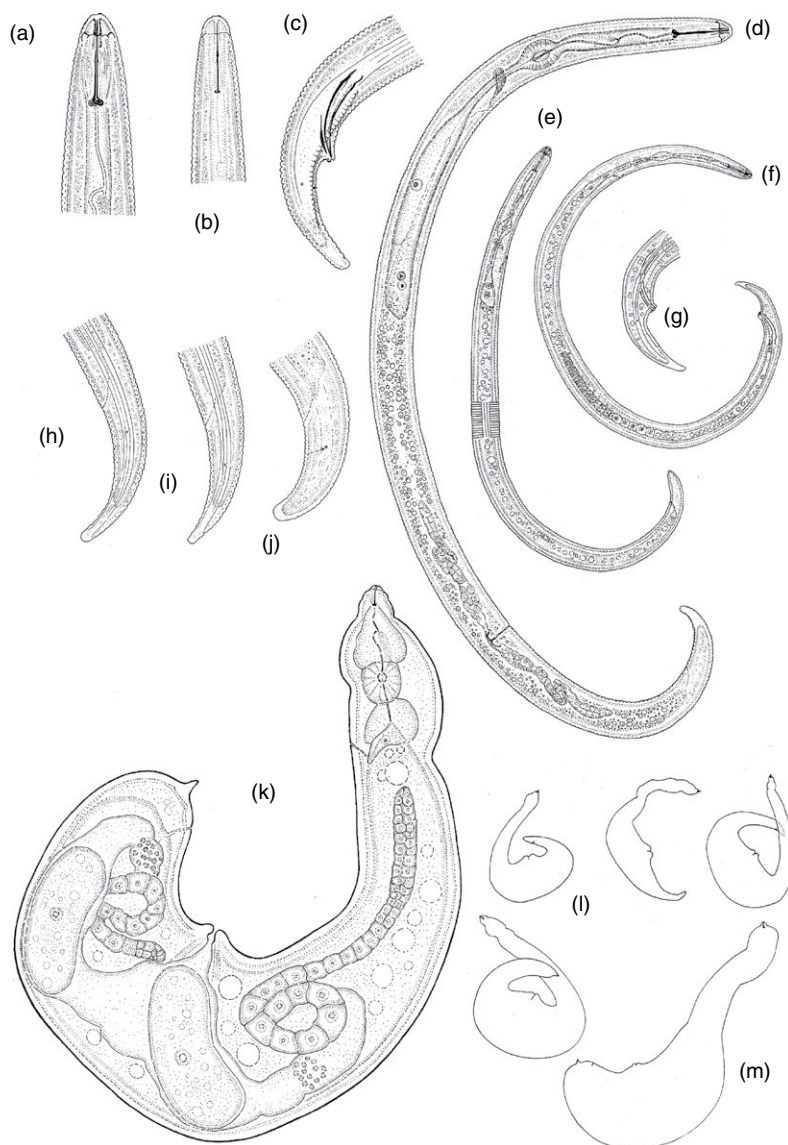


Fig. 2.15. *Rotylenchulus reniformis* (a) female labial region; (b) male labial region; (c and g) male tail; (d) entire immature female; (e) entire juvenile; (f) entire male; (h and i) immature female tails; (j) juvenile tail; (k and m) entire mature females. *Rotylenchulus parvus* (l) entire mature females. Line drawings are for illustrative purposes only and are not to scale.

Useful literature

- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 322–330.
- Van den Berg, E. and Quénévér, P. (2012) Hoplolaimidae. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 251–297.
- Van den Berg, E., Palomares-Rius, J.E., Vovlas, N., Tiedt, L.R., Castillo, P. and Subbotin, S.A. (2016) Morphological and molecular characterisation of one new and several known species of the reniform nematode, *Rotylenchulus* Linford & Oliveira, 1940 (Hoplolaimidae: Rotylenchulinae), and a phylogeny of the genus. *Nematology* 18, 67–107.

***Heterodera* Schmidt, 1871 (Tylenchina, Heteroderidae) = *Afenestrata*, *Bidera*, *Ephippiodera* (Fig. 2.16a, b, d–f and h–j)**

MORPHOLOGY: sexually dimorphic. **Female:** obese, **lemon-shaped**, approximately 300 μm in diameter with a distinct neck and partially enclosed either in root tissue or in the soil. **Oral**

disc squarish, strongly offset. Vulva subterminal, near anus. Cuticle thick, whitish at first, but tanning to a brownish-black colour as cyst matures. **Eggs retained within protective cyst.** **Vulva and anus located on a terminal cone with two translucent areas, the fenestrae, on either side of vulval slit.** Two convoluted genital tracts. **In young females, excretory**

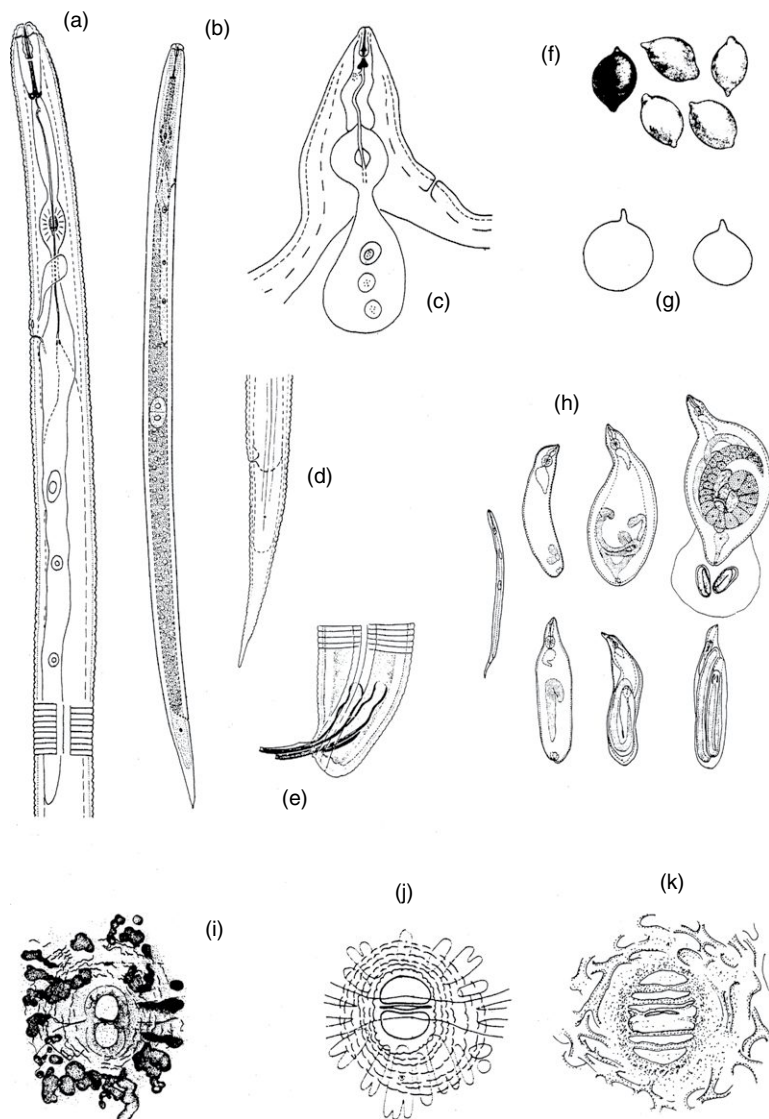


Fig. 2.16. *Globodera rostochiensis* (c) female anterior region; (g) entire cysts; (k) perivulval area. *Heterodera avenae* (e) male tail; (f) cysts; (i) perivulval area. *Heterodera glycines* (j) perivulval area. *Heterodera oryzae* (d) juvenile tail. *Heterodera sacchari* (a) J2 pharynx; (b) J2 infective juvenile. *Heterodera schachtii* (h) developmental stages. Line drawings are for illustrative purposes only and are not to scale.

pore visible at level of, or posterior to, median bulb valve plates. Male: vermiform with body often twisted through 180° on heat relaxation; found free in soil. Stylet and skeleton of labial region robust. Tail short, hemispherical. Spicules opening subterminally. No bursa. Juvenile (J2): vermiform, 450–600 µm long with stylet and labial region skeleton robust. Tail conical with hyaline area starting well before tail terminus.

***Globodera* Skarbilovich, 1959 (Tylenchina, Heteroderidae) (Fig. 2.16c, g and k)**

MORPHOLOGY: similar to *Heterodera* except for the **globose cyst. Vulva and anus not elevated on a terminal cone and vulval slit surrounded by a single, circular fenestra.**

BIOLOGY: in most species, all the eggs are retained within the mature cyst, although in some species a voluminous external egg mass is present (e.g. *Heterodera oryzae*). Eggs often hatch in response to root exudates from a host plant, although other hatching factors can be involved. The J2 emerges from the egg, invades a root and

induces a feeding site composed of syncytial nurse cells. Root galling is not induced. The J2 swells and moults three times to form the adult female, which enlarges rapidly, the posterior region bursting through the root epidermis. Males are more commonly produced when food is in short supply. They assume a vermiform state within the J4 cuticle before burrowing out of the root into the soil. Females produce several hundred eggs and, after death, the cuticle of the female tans to form a protective cyst.

MAJOR SPECIES: *H. avenae*, *H. cajani*, *H. ciceri*, *H. glycines*, *H. latipons*, *H. sacchari*, *G. pallida*, *G. rostochiensis*.

DISTRIBUTION: although the majority of *Heterodera* species are temperate in distribution, some species are present in tropical or subtropical crops, whereas *Globodera* species tend to be confined to cooler regions.

CONFUSABLE GENERA: *Afenestrata*, *Cactodera*, *Punctodera*. The J2 infectives can be confused with those of other genera of the same family and share some similarities with those of *Meloidogyne*.

Useful literature

- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 387–410.
- Subbotin, S.A. and Franco, J. (2012) Cyst nematodes. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 299–357.
- Subbotin, S.A., Mundo-Ocampo, M. and Baldwin, J.B. (2010) *Systematics of Cyst Nematodes (Nematoda: Heteroderinae)*. In: Hunt, D.J. and Perry, R.N. (series eds) *Nematology Monographs and Perspectives*. Volumes 8A, 8B. Brill, Leiden, the Netherlands, 351 pp. (8A), 512 pp. (8B).

***Meloidogyne* Goeldi, 1887 (Tylenchina, Meloidogynidae) = *Hypsoperine* (Fig. 2.17)**

MORPHOLOGY: **sexually dimorphic.** Female: embedded in root tissue, globose, 0.3–0.7 mm in diameter **with a slender neck. Vulva subterminal near anus.** Cuticle whitish, thin, annulated. Stylet short, moderately sclerotized. Labial region skeleton weak. **Excretory pore located anterior to median bulb valve plates** and often near to stylet base. Two convoluted genital tracts. Eggs **deposited outside body in a gelatinous matrix.** Male: vermiform, free-living in

soil, 1–2 mm long. **Body usually twisted through 180° along its length on heat relaxation. Stylet and labial region skeleton robust. Tail short,** hemispherical. Spicules robust. Bursa absent. Juveniles (J2): slender, vermiform, about 450 µm long. Stylet and labial region skeleton weakly sclerotized. **Tail conical with hyaline portion starting near tail tip.**

BIOLOGY: in most species, the eggs are retained within a gelatinous matrix outside the swollen female body. On hatching, the J2 invades a host root and induces a trophic

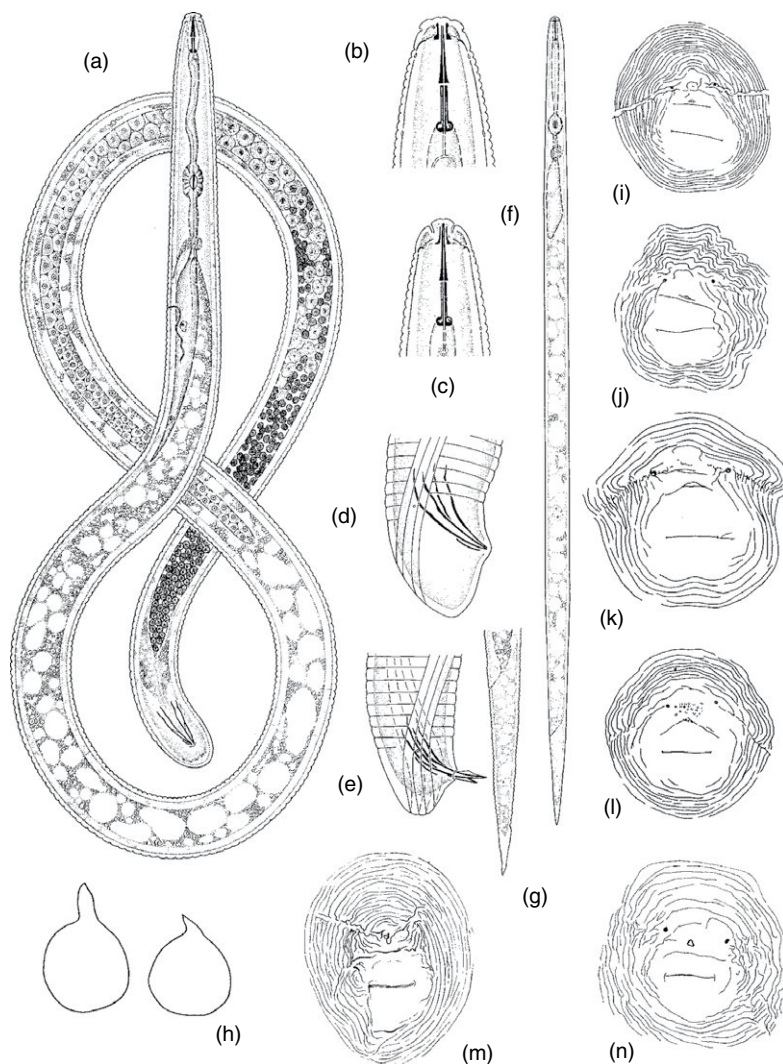


Fig. 2.17. *Meloidogyne incognita* (a) entire male; (b and c) male labial region; (d and e) male tail; (f) entire infective juvenile (J2); (g) J2 tail; (h) mature females. Perineal patterns (i) *Meloidogyne javanica*; (j) *Meloidogyne incognita*; (k) *Meloidogyne arenaria*; (l) *Meloidogyne hapla*; (m) *Meloidogyne graminicola*; (n) *Meloidogyne exigua*. Line drawings are for illustrative purposes only and are not to scale.

system of giant cells. Cortical cells are induced to multiply to form the characteristic gall. The remainder of the life cycle is similar to that of *Heterodera*/*Globodera*, except that in most species the females do not normally burst out of the root because of the surrounding gall tissue.

MAJOR SPECIES: *M. arenaria*, *M. exigua*, *M. graminicola*, *M. hapla*, *M. incognita*,

M. javanica, *M. enterolobii* (= *M. mayaguensis*), *M. ethiopica*.

DISTRIBUTION: widely distributed throughout the tropical and subtropical regions.

CONFUSABLE GENERA: *Nacobbus*. The J2 infective stage might be confused with those of *Heterodera*/*Globodera*, but has weaker cephalic sclerotization, a less robust stylet and a shorter hyaline region in the tail.

Useful literature

- Hunt, D.J. and Handoo, Z.A. (2009) Taxonomy, identification and principal species. In: Perry, R.N., Moens, M. and Starr, J.L. (eds) *Root-knot Nematodes*. CAB International, Wallingford, UK, pp. 55–97.
- Hunt, D.J. and Handoo, Z.A. (2012) Root-knot nematodes. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds). *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 359–410.
- Karssen, G., Wesemael, W. and Moens, M. (2013) Root-knot nematodes. In: Perry, R.N. and Moens, M. (eds) *Plant Nematology*, 2nd edn. CAB International, Wallingford, UK, pp. 74–108.
- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 369–382.

***Criconemoides* Taylor, 1936, and *Mesocriconema* Andrásy, 1965 (Tylenchina, Criconematidae) = *Criconemella*, *Xenocriconemella*, *Madinema*, *Seshadriella*, *Neobakernema*, *Crossonemoides* (Fig. 2.18)**

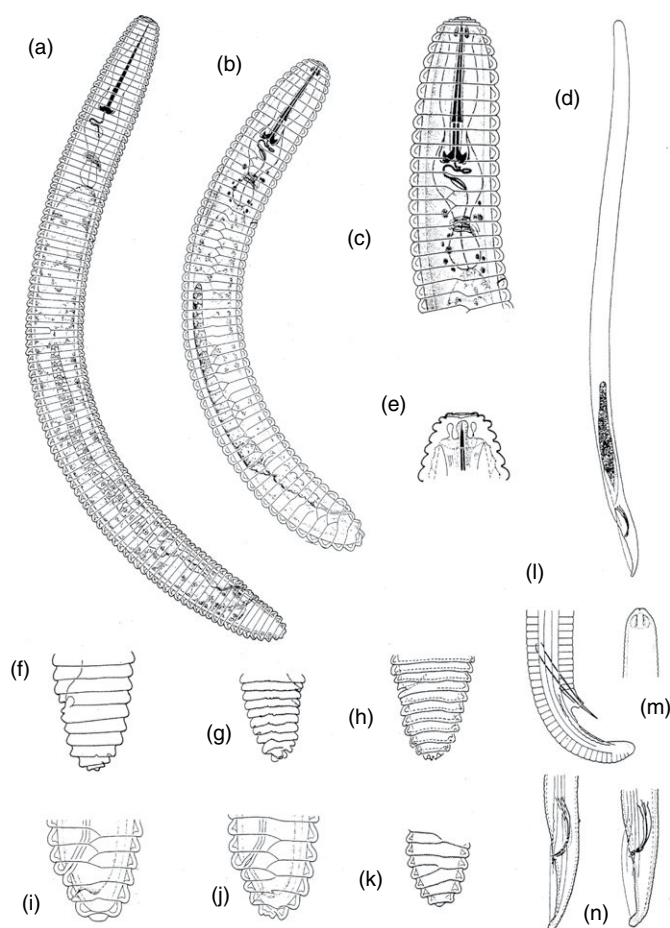


Fig. 2.18. *Criconemoides pseudohercyniensis* (= *Criconemoides morgensis*) (d) entire male; (e) female labial region; (g) female tail; (n) male tails. *Mesocriconema onoense* (h) female tail. *Mesocriconema sphaerocephalum* (b) entire female; (c) female pharyngeal region; (i and j) female tails. *Mesocriconema xenoplax* (a) entire female; (f) female tail; (k) juvenile tail; (l) male tail; (m) male labial region. Line drawings are for illustrative purposes only and are not to scale.

MORPHOLOGY: strong sexual dimorphism. Female: body 0.20–1 mm long, stout, dying straight or slightly curved, with rounded anterior end, and rounded to conical posterior part. **Cuticle provided with 42–200 prominent, retrorse annuli**, with a **smooth or finely crenate posterior margin**. Labial area not well separated from rest of body, marked by one or two thinner annuli, submedian lobes usually present and separate (*Mesocriconema*) or, if present, not separate (*Criconemoides*). **Stylet strong, basal knobs with a forwardly directed process** (= anchor shaped). **Pharynx with a strong median bulb that is fused with the procorpus; glands forming a small posterior bulb. Vulva posterior, lips open (*Mesocriconema*) or closed (*Criconemoides*). One genital tract, extending anteriorly.** Spermatheca laterally situated. Male: body slender and short. Anterior end rounded. No stylet; pharynx degenerate. Spicule short, slightly curved. Bursa weakly developed, exceptionally absent. Tail pointed. Juveniles: resembling female. Annuli smooth to finely crenate (exceptionally with a row of scales) on posterior margin.

BIOLOGY: migratory ectoparasites on perennial crops, trees and vines. Males non-feeding. Most species are parthenogenetic. Only a few species have been proved to be harmful. Found in all geographic areas.

MAJOR SPECIES: *M. axeste*, *M. onoense*, *M. rusticum*, *M. sphaerocephalum*, *M. xenoplax*.

CONFUSABLE GENERA: each other, *Criconema*, *Discocriconemella*, *Hemicriconemoides*.

TAXONOMIC NOTE: species of *Criconemoides*/*Mesocriconema* have been commonly placed in *Macroposthonia* and/or *Criconemella*. This situation can be confusing and must be borne in mind when consulting the literature. The International Commission on Zoological Nomenclature (ICZN) decreed *Criconemoides* to be a valid generic name and suppressed *Macroposthonia*. *Mesocriconema* can be distinguished from *Criconemoides sensu stricto* by certain morphological details (see above) and molecular phylogeny. Major pest species occur in *Mesocriconema*.

Useful literature

- Cid del Prado Vera, I. and Talavera, M. (2012) Criconematoidea. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 479–519.
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- Siddiqi, M.R. (2000) *Tylenchida Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK, pp. 509–579.

***Hemicycliophora* de Man, 1921 (Tylenchina, Criconematidae) = *Aulosphora*, *Colbranium*, *Loofia* (Fig. 2.19a–h)**

MORPHOLOGY: strong sexual dimorphism. Female: body straight, or slightly ventrally curved, 0.6–1.9 mm long, stout. Anterior end rounded. Posterior end pointed, more rarely rounded. **Cuticle with detached sheath** (= 'double' cuticle); external layer marked by numerous (up to 400) prominent annuli; annuli **not retrorse**. No true lateral field, but cuticle may be variously ornamented (longitudinal lines, squares, dots, scratches, etc.). Labial area

not separated from body, marked by 2–3 annuli. **Stylet strong, long, with rounded basal knobs. Pharynx with strong median bulb fused with procorpus; glands forming a small terminal bulb abutting intestine. Vulva posteriorly situated. One anteriorly directed genital tract;** spermatheca lateral. Anus and rectum vestigial. Post-vulval part generally conical with pointed terminus, more rarely cylindrical with rounded extremity. Male: slender, with simple cuticle (outer layer not detached). **No stylet. Pharynx degenerate. Spicules strong, semi-circular to hook shaped. Bursa adanal, well developed.** Tail

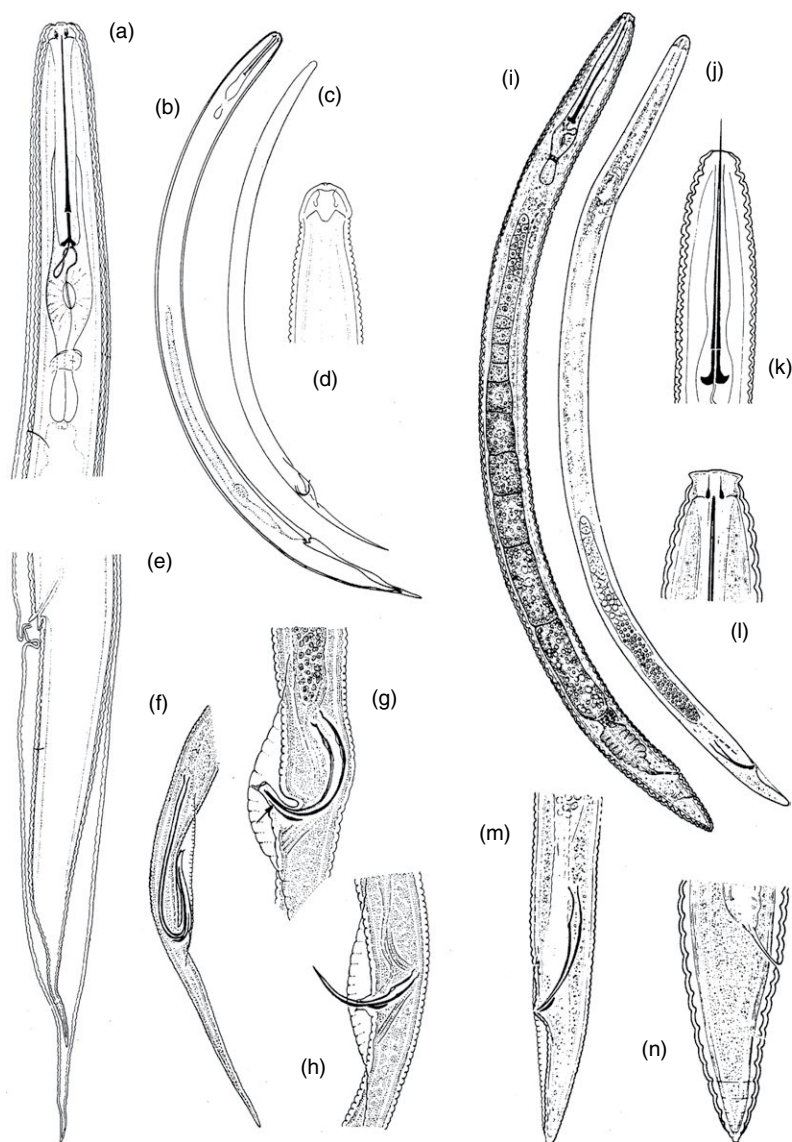


Fig. 2.19. *Hemicycliophora chathamii* (a) female pharynx; (b) entire female; (c) entire male; (d) male labial region; (e) female posterior region; (g) male tail. *Hemicycliophora penetrans* (f) male tail. *Hemicycliophora thienemanni* (h) male tail. *Hemicriconemoides mangiferae* (i) entire female; (j) entire male; (l) female labial region; (m) male tail; (n) female tail. *Hemicriconemoides chitwoodi* (k) female stylet. Line drawings are for illustrative purposes only and are not to scale.

long, conical, often angled ventral to body axis.
Juveniles: resembling female.

BIOLOGY: as for *Criconemoides*.

MAJOR SPECIES: *H. arenaria*, *H. parvana*, *H. typica*.

CONFUSABLE GENERA: *Caloosia*, *Hemicriconemoides*.

Useful literature

- Chitambar, J.J. and Subbotin, S.A. (2014) *Systematics of the Sheath Nematodes of the Superfamily Hemicycliophoroidea*. In: Hunt, D.J. and Perry, R.N. (series eds) *Nematology Monographs and Perspectives*, Vol 10. Brill, Leiden, the Netherlands, 732 pp.
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***Hemicriconemoides* Chitwood & Birchfield, 1957 (Tylenchina, Criconematidae) (Fig. 2.19i–n)**

MORPHOLOGY: strong sexual dimorphism. Female: similar in many ways to *Hemicycliophora*, but shorter (usually c.0.5 mm long), with fewer annules and with very closely adpressed 'double' cuticle. Stylet knobs with anteriorly directed

processes. Tail short, conoid. Juveniles resembling female, but posterior margin of body annuli ornamented with scales or short denticles.

BIOLOGY: similar to *Criconemoides*.

MAJOR SPECIES: *H. cocophillus*, *H. strictathecatus* (= *H. mangiferae*).

CONFUSABLE GENERA: *Caloosia*, *Hemicycliophora*.

Useful literature

- Cid del Prado Vera, I. and Talavera, M. (2012) Criconematoidea. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 479–519.
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***Tylenchulus* Cobb, 1913 (Tylenchina, Tylenchulidae) (Fig. 2.20)**

MORPHOLOGY: **sexually dimorphic**. Immature female (free in soil): body vermiform, ventrally curved posteriorly, small (<0.5 mm). Labial region rounded, continuous with body contour. **Labial sclerotization weak**. Stylet of medium development with rounded basal knobs. **Pharynx with strong median bulb not well separated from procorpus; glands forming a basal bulb. Vulva very posteriorly**

situated; genital tract single, anteriorly outstretched. Excretory pore located very posteriorly and only slightly anterior to vulva. Tail conical. No anus or rectum. Mature female: **anterior part embedded in root tissue**, irregular, slender, with thin cuticle. Posterior part, bursting out of root, swollen with very thick cuticle and a pointed post-vulval section; **excretory pore and vulva very posterior**. Excretory cell well developed, producing a gelatinous matrix. Genital tract convoluted, with several eggs. **No anus or rectum.**

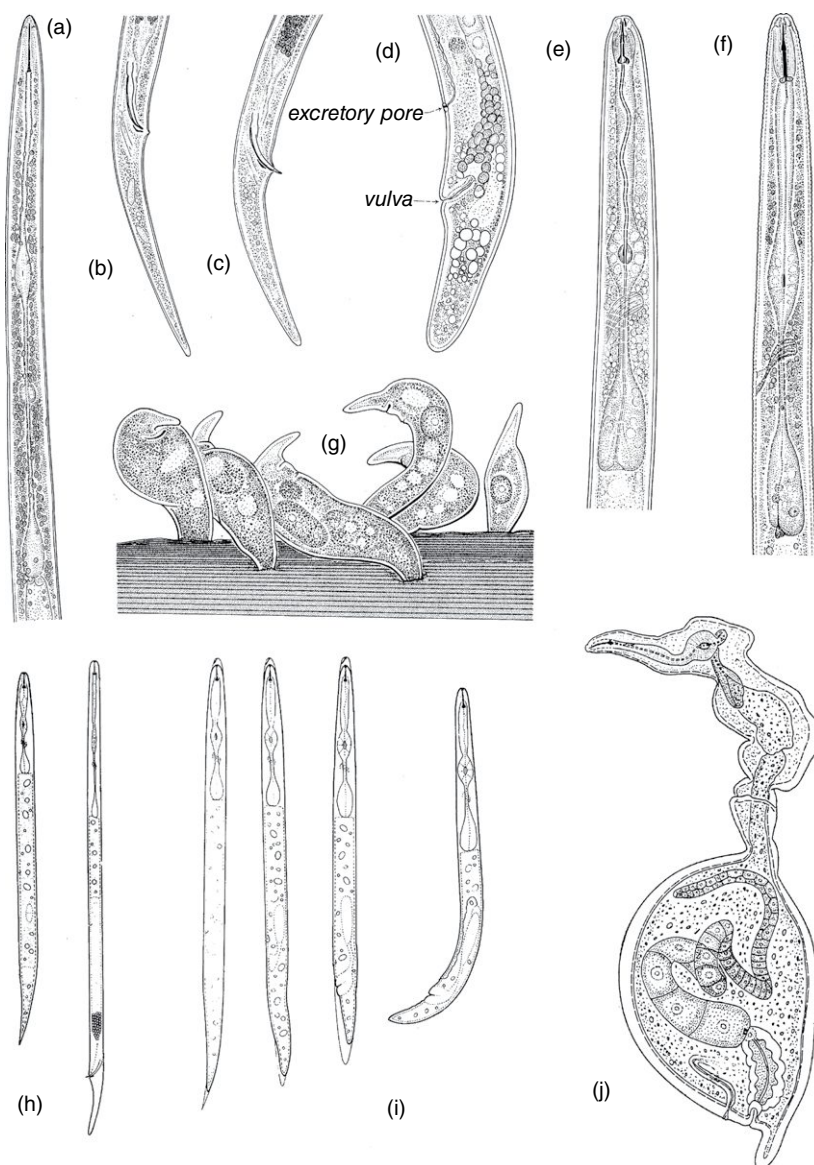


Fig. 2.20. *Tylenchulus semipenetrans* (a) male pharynx; (b and c) male tails; (d) female posterior region; (e) immature female pharynx; (f) juvenile pharynx; (g) mature females attached to root; (h) development of male; (i) development of female; (j) entire female. Line drawings are for illustrative purposes only and are not to scale.

Male: body vermiform, short and slender. **Cephalic sclerotization, stylet and pharynx reduced.** Spicules slightly curved. **No bursa.** Tail conical, pointed. Juvenile: body vermiform. Labial sclerotization, stylet and pharynx similar to those of immature females. Tail long, pointed. Genital primordium differently shaped in male and female juveniles from J2 onwards.

BIOLOGY: the eggs are contained in a gelatinous matrix produced by the secretory/excretory cell. After hatching, male juveniles moult to the adult without feeding, while female juveniles feed on cortical cells. The immature female penetrates deeper into the root, the anterior end penetrating deep into the cortex while the posterior section, which becomes obese, remains

outside the root. A highly sophisticated system of trophic nurse cells is initiated around the female labial region. (Note: a heavily infested citrus root, when rinsed carefully in water, retains a collar of earth adhering to the gelatinous egg sacs underneath.)

MAJOR SPECIES: *Tylenchulus semipenetrans*.

DISTRIBUTION: found almost everywhere that citrus is grown on any scale, and often causing a severe disease called 'slow decline'.

CONFUSABLE GENUS: *Trophotylenchulus*.

Useful literature

- Cid del Prado Vera, I. and Talavera, M. (2012) Criconematoidea. In: Manzanilla-López, R.H. and Marbán-Mendoza, N. (eds) *Practical Plant Nematology*. Biblioteca Básica de Agricultura, Guadalajara, Mexico, pp. 479–519.
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***Xiphinema* Cobb, 1913 (Dorylaimina, Longidoridae) (Fig. 2.21c, d and g–m)**

MORPHOLOGY: slender nematodes, 1.3–5 mm long. Labial region continuous or offset. **Amphidial aperture a broad slit leading back to a funnel-shaped pouch.** Stylet very long (60–250 µm), consisting of a needle-like **odontostyle with a forked base** attached to an **odontophore with three prominent basal flanges.** Stylet guide appearing tubular with the 'guide ring' located in posterior half of odontostyle. Pharynx consisting of a long, narrow procarpus and a short, glandular bulb. Female: vulva usually located at 40–50% of body length, but may be more anterior. Usually two genital tracts present, but in some species the anterior tract is non-functional (mono-opisthodelphic or pseudo-mono-opisthodelphic) and reduced to varying degrees, or even entirely absent, in which case the vulva is more anteriorly located at c.25% of body length. Tail very variable from short and rounded to long filiform. Male: spicules very powerful, arcuate. Ventral supplements forming a pre-cloacal row.

***Longidorus* Micoletzky, 1922 (Dorylaimina, Longidoridae) (Fig. 2.21a, e and n)**

MORPHOLOGY: similar to *Xiphinema* but body thinner and may be up to 11 mm long. **Amphids**

pouch-like and opening via a minute, inconspicuous pore. Odontostyle/odontophore junction not forked, odontophore lacking flanges and odontostylet less strongly sclerotized. **Guide ring located in anterior half of odontostyle.**

***Paralongidorus* Siddiqi, Hooper & Khan, 1963 (Dorylaimina, Longidoridae) = *Siddiqia*, *Inagreius*, *Longidoroides* (Fig. 2.21b and f)**

MORPHOLOGY: similar to *Longidorus*, but **amphidial pouch stirrup-shaped** and amphidial **aperture broad and slit-like**, as in *Xiphinema*.

BIOLOGY: long-lived, migratory ectoparasites attacking a wide variety of hosts. The favoured point of attack is at or near the root tip, resulting in hooked root tips and/or terminal galls. Attacked root systems are stunted, lack developed laterals and show necrosis at the feeding sites. *Xiphinema* tends to be more abundant under woody hosts, whereas *Longidorus* and *Paralongidorus* are more common under non-woody plants, particularly grasses and cereals. Greatest populations are found below 30 cm. With few exceptions, sandy soils support higher populations than heavier soils. Some species have been shown to be virus

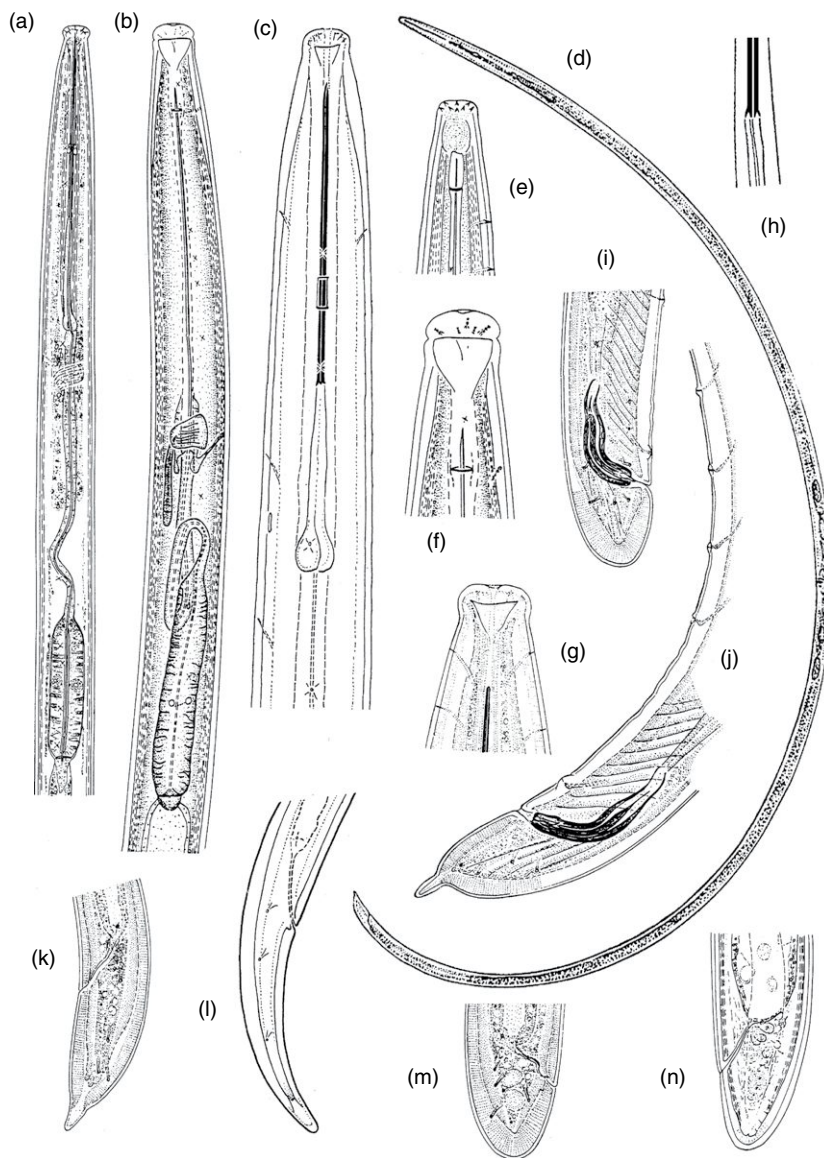


Fig. 2.21. *Longidorus fursti* (a) pharynx; (n) female tail. *Longidorus elongatus* (e) labial region. *Paralongidorus natalensis* (b) pharynx; (f) labial region. *Xiphinema heynsi* (i) male tail; (m) female tail. *Xiphinema mammatum* (j) male tail. *Xiphinema neobasiri* (d) entire female; (g) labial region; (k) female tail. *Xiphinema savanicola* (c) pharynx; (h) odontostyle/odontophore junction; (l) female tail. Line drawings are for illustrative purposes only and are not to scale.

vectors. Reproduction is amphimictic or parthenogenetic.

MAJOR SPECIES: *X. americanum* group, *X. index*, *X. elongatum*, *L. africanus*, *L. laevicapitatus*, *P. australis*.

DISTRIBUTION: *Longidorus* is found mainly in cooler areas, while *Xiphinema* and *Paralongidorus* are more tropical.

CONFUSABLE GENERA: each other, *Paraxiphidorus*, *Xiphidorus*.

Useful literature

- Coomans, A., Huys, R., Heyns, J. and Luc, M. (2001) Character analysis, phylogeny, and biogeography of the genus *Xiphinema* Cobb, 1973 (Nematoda, Longidoridae). *Annales du Muséum Royal de l'Afrique Centrale (Zoologie)*, Tervuren, Belgium, 287, 1–239.
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- Hunt, D.J. (1993c) *Aphelenchida, Longidoridae and Trichodoridae: Their Systematics and Bionomics*. CAB International, Wallingford, UK.

***Trichodorus* Cobb, 1913 (Diphtherophorina, Trichodoridae) (Fig. 2.22f–l)**

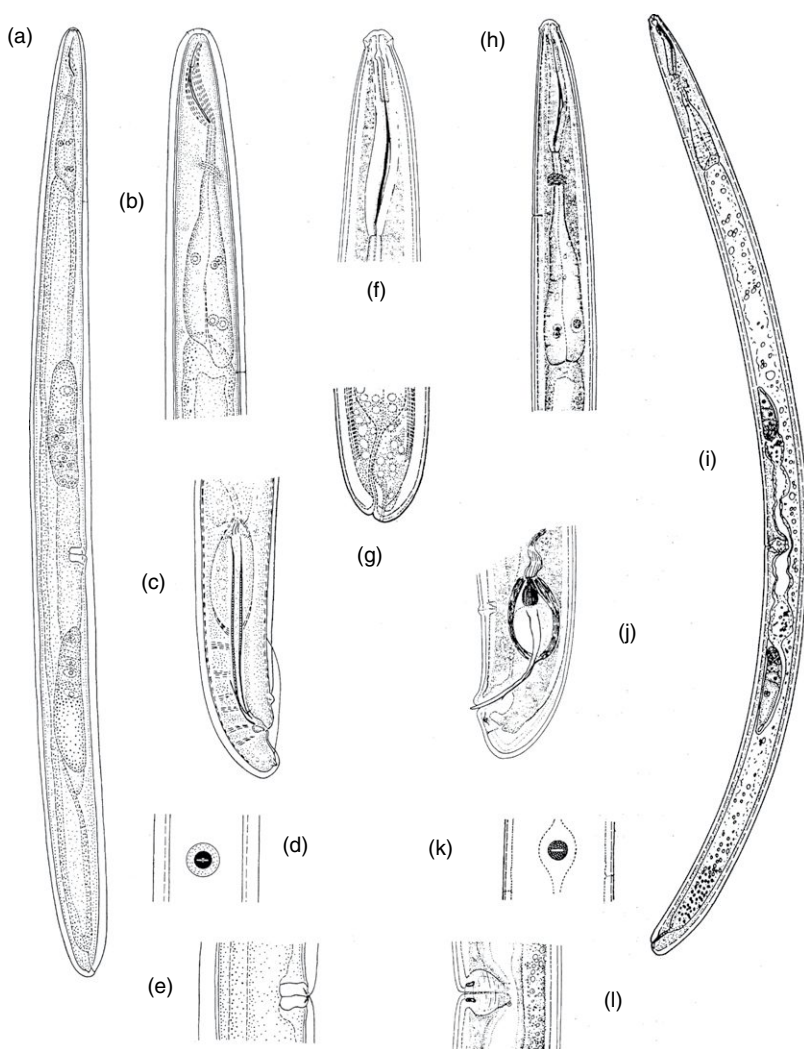


Fig. 2.22. *Nanidorus minor* (a) entire female; (b) pharynx; (c) male tail; (d) vulva, ventral view; (e) vulva, lateral view. *Trichodorus primitivus* (f) labial region; (h) pharynx; (j) male tail; (l) vulva lateral view. *Trichodorus similis* (g) female tail; (k) vulva, ventral view. *Trichodorus viruliferus* (i) entire female. Line drawings are for illustrative purposes only and are not to scale.

MORPHOLOGY: **body stout, cigar shaped**, 0.8–1.2 mm long. Cuticle smooth. Labial region continuous with body contour; papillae prominent. **Onchiostyle** (= stylet) **tripartite, curved**. Pharynx **slender anteriorly with a posterior bulboid expansion**. Female: vulva median with **strong vaginal sclerotization, vagina well developed, extending into body for about half its diameter, one pair of lateral body pores present within one body diameter of vulva**. Two genital tracts. **Tail rounded, very short**; anus almost terminal. Male: spicules arcuate, gubernaculum present. **Protractor muscles conspicuous, of unusual form** and encapsulating spicule shafts. Ventral supplements present, **bursa usually absent or very small**.

***Paratrichodorus* Siddiqi, 1974 and *Nanidorus* Siddiqi, 1974 (Diphtherophorina, Trichodoridae) (Fig. 2.22a–e)**

MORPHOLOGY: very similar to *Trichodorus* but **cuticle markedly swelling in response to acidic fixatives**. Female: **vulva in form of a short longitudinal slit (*Paratrichodorus*) or short transverse slit (*Nanidorus*), with weak vaginal sclerotization, vagina weakly developed, extending into body for about one-third of its diameter. No lateral body**

pores within one body diameter of vulva. Caudal pores present (*Paratrichodorus*) or absent (*Nanidorus*). Male: spicule protractor muscles inconspicuous. Bursa present. Pre-cloacal supplements 3 (*Paratrichodorus*) or 1 (*Nanidorus*).

BIOLOGY: ectoparasitic on the roots of perennial and woody plants. The main area of attack is just behind the root tip, thereby restricting root elongation. The root tip is then attacked, as are the developing lateral root initials, resulting in the characteristic 'stubby root' system. Both genera are more common in light or sandy soils, and highest densities tend to occur at depths of 30–40 cm. Some species are known to be virus vectors and it is likely that the other species are potential vectors.

MAJOR SPECIES: *T. primitivus*, *T. similis*, *T. viruliferus*, *N. minor*, *P. pachydermus*.

DISTRIBUTION: worldwide. *Trichodorus* tends to occur more in temperate regions while *Paratrichodorus* and *Nanidorus* (now generally accepted as a valid genus rather than being viewed as a junior synonym of *Paratrichodorus*) are more tropical.

CONFUSABLE GENERA: *Monotrichodorus* (only one female genital tract) and each other.

Useful literature

- Decraemer, W. (1995) *The Family Trichodoridae: Stubby Root and Virus Vector Nematodes*. Kluwer Academic Publishers, Dordrecht, the Netherlands.
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S.A. 6, 155–169 (Fig. 2.13a and c–g); 9, 267–295 (Fig. 2.13k–m). *Phytopathology*: Raski, D.J. 40, 135–152 (Fig. 2.16h). *Phytophylactica*: Jacob, P.J.F. and Heyns, J. 14, 169–178 (Fig. 2.21a, b, f and n). (Reproduced under South Africa Government Printer's copyright authority 9017 of 5 July 1989.) *Proceedings of the Helminthological Society of Washington*: Dasgupta, D.R., Raski, D.J.

and Sher, S.A. 35, 169–192 (Fig. 2.15a–m); Sher, S.A. 35, 219–237 (Fig. 2.10a–g and i–p). *Revue de Nématologie*: Luc, M. and Southey, J.F. 3, 243–269 (Fig. 2.1e, n; Fig. 2.21m); Siddiqi, M.R. 2, 51–64 (Fig. 2.1e and n; Fig. 2.21g, i–k and m); 3, 179–199 (Fig. 2.19f–h). *Soil and Freshwater Nematodes*: Goodey, J.B. Methuen, London. 544 pp. (Fig. 2.20j). *Systematic Parasitology*: Orton Williams, K.J. 8, 207–214 (Fig. 2.19a–e and g). (Reprinted by permission of Kluwer Academic Publishers.) *United States Department of Agriculture* (Fig. 2.10h; After N.A. Cobb, 1915). All other illustrations courtesy of CAB International.

Note

¹ All figure references in this section refer to Fig. 2.1.

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3 Nematode Ecology and Soil Health

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Soil and Plant Health

We understand intuitively the concept of soil health but often find it difficult to articulate. Generally, we are able to define the unhealthy condition more clearly than the healthy state of individuals, populations or ecosystems. Sorauer *et al.* (1908) defines plant health as that condition where the physiological functions are within an optimal range and support normal development, biological processes and survival. The same definition can be extended to animal and human health and, when considered in terms of their functions and services, to ecosystems. Many essential ecosystem functions and services are performed in the soil and provide basic support of life on earth. They sustain plant and animal productivity, provide a reservoir for genetic diversity and are responsible for the remediation of water and air quality (Karlen *et al.*, 1997). The functions are supported by myriad soil organisms that perform the essential processes of decomposing organic materials, mineralizing organic molecules, sequestering and redistributing minerals, and regulating the abundance of pest species. Those and other functions of the soil ecosystem underpin the production of food, fibre, fuel and construction materials.

Anthropogenic manipulation of the soil environment to enhance agricultural productivity in support of the human population is a large contributor to the degradation of soil quality.

Likewise are the pollution and contamination effects of many industrial processes and the complex waste streams engendered by human lifestyles and social configurations (Pimentel *et al.*, 1997). In low-input, arguably more sustainable, systems, the soil provides the necessary resources to support the physiological processes of plants. Hence, plant health is linked to soil health. We uncouple that linkage in modern agricultural practices by providing the necessary resources from external sources and modifying negative functions of the soil ecosystem by using pesticides. If we wish to transition to sustainable production systems that minimize inputs of pesticides, fertilizers and fossil fuels, the linkage between plant health and soil health must be re-established, maintained and nurtured.

In a recent review, Reeve *et al.* (2016) describe the mechanisms by which soil condition determines plant health to include: (i) the physical component, through the effects of soil structure, porosity and bulk density on rooting depth, water availability and aeration; (ii) the chemical properties, which provide plant nutrients but which may also present a saline or otherwise toxic environment; and (iii) the biological component, which has both positive and negative effects on plant performance. In agroecosystems, soil management alters all soil components, and thereby determines both soil and plant health. Unravelling the effects of different management techniques on soils is, thus, a key

process in the establishment, maintenance and assessment of soil and plant health in sustainable agricultural systems. Nematode-based ecological indicators are proving to be useful tools in such endeavours. Until recently, soil nematodes have been studied largely as pest organisms (see, for example, other chapters in this volume), disrupting normal developmental and physiological process of plants. However, the vast majority of nematode species in soils are not parasites of plants but rather contribute variously to the beneficial and essential ecosystem services of soils.

Soil Ecosystem Structure: Abiotic and Biotic Components

Typically, soils are considered to possess physical, chemical and biological attributes. The soil physical structure is determined by the nature of the parent material and by breakdown processes such as weathering, but is influenced too by chemical and biological processes. Each soil has characteristic sizes of particle, particle aggregations and homogeneity of strata. That physical structure can be altered by the biological inhabitants and by anthropogenic manipulations and modifications. The soil chemical constitution, on the other hand, is a product of the degradation of parent material and the effects of climatic weathering, the activities of the biological inhabitants and amendment by pesticides and fertilizers. The soil biological

component is perhaps most complex and least explored in terms of the diversity and abundance of its species assemblages and of their functional complexity. The three components of the soil influence each other, with the interaction of the physical and chemical components regulating the nature and activities of the biological component (Table 3.1.).

The Soil Environment as a Habitat for Organisms

The soil provides a multidimensional matrix of differing microhabitats with a wide range of space–time–trait characteristics that provide habitat for a great variety of organisms. The differences among microhabitats are determined by the particulate, porosity and stratification configuration of soil, from the disruption of its symmetry by roots, burrows and impervious zones, from the effects of physical and chemical gradients, and from diurnal, seasonal and stochastic climatic events. At the nucleus of each microhabitat is, commonly, a resource of some form that is available for exploitation by the combinations of organisms of different species with traits and trophic habits suitable for that space. Consequently, bottom-up (e.g. nutrient availability) and top-down (e.g. predation) forces, combined with current ambient conditions, drive waves of biological activity and ecosystem functions in each microhabitat.

Table 3.1. Main soil physical and chemical attributes and their effect on ecosystem functions and services derived from the functioning of its biological component. (From Karlen *et al.*, 1997)

Soil attributes	Ecosystem functions and services affected
Organic matter	Nutrient cycling, pesticide and water retention, soil structure, antagonistic potential toward plant disease and nematodes
Infiltration	Runoff and leaching potential, plant water-use efficiency, erosion potential
Aggregation	Soil structure, erosion resistance, crop emergence, infiltration
pH	Nutrient availability, pesticide absorption and mobility
Microbial biomass	Biological activity, nutrient cycling, capacity to degrade pesticides, and both positive and negative effects on plant health
Forms of nitrogen	Availability to crops, leaching potential, mineralization and immobilization rates
Bulk density	Plant root penetration, water- and air-filled pore space, biological activity
Topsoil depth	Rooting volume for crop production, water and nutrient availability
Conductivity or salinity	Water infiltration, crop growth, soil structure
Available nutrients	Capacity to support crop growth, environmental hazard

Nematodes as Bioindicators: Trophic Levels and Functional Guilds

The measurement of ecosystem health, and soil health specifically, requires consideration of the condition of a number of criteria, including the nature, condition and function of the complex organism assemblages that compose the soil metacommunity. Typically, and in the absence of integrative assessment methods, measures of soil health are most conveniently provided by indicators, either of soil species composition and/or of several ecosystem functions, that allow broader inference of the structure (that is, the composition of the assemblage of organisms) and functions of the soil system. Many indicators of soil condition have been used, but among biological indicators the composition of the nematode assemblage is frequently used. Nematodes are abundant, taxonomically and functionally diverse, occur in all soil and aquatic systems and differ in their physiological, developmental, behavioural and food preference characteristics (Bongers and Ferris, 1999).

Soil nematode functional diversity is reflected primarily by the variety of nematode trophic groups and the differences among species in sensitivity to enrichment and perturbation. The potential of nematodes as bioindicators is

based in that diversity, and their use has been facilitated greatly by the proposal of the colonizer–persister (cp) series that recognized five groups ranging from extreme r- to extreme K-strategists (Bongers, 1990; Bongers and Bongers, 1998). ‘Colonizer’ nematodes, at the lower end of the cp scale (cp groups 1 and 2) are considered opportunists and therefore indicate resource availability or enhancement; ‘persister’ nematodes at the high end of the scale (cp-4 and cp-5) indicate system stability, food web complexity and connectance among organisms (Table 3.2). The weighted prevalence of each cp group in the nematode community is the basis of the maturity index (MI), which has expanded into a family of indices as researchers recognized applications in addressing a variety of environmental assessments (Bongers, 1990; Yeates, 1994; Neher and Campbell, 1996; Bongers *et al.*, 1997; Bongers and Ferris, 1999). Besides being categorized as cp groups, soil nematodes are commonly classified into five trophic groups that represent the most common of the eight trophic habits described by Yeates *et al.*, 1993. A matrix of functional guilds is formed by the intersection of the two classifications (Table 3.2), where a guild is a group of species that exploit the same resource and contribute to the same ecosystem function. The matrix of functional guilds provides a base

Table 3.2. Ecological characteristics of nematode colonizer–persister (cp) and trophic groups. (From Bongers, 1990; Yeates *et al.*, 1993; Ferris *et al.*, 2001.)

Cp groups

Cp-1: short generation time. Large gonad:body ratio. Many small eggs. Rapid response to resource enrichment. High metabolic activity. Survive adverse conditions through tolerance or dormancy.

Cp-2: short generation time. Relatively high reproduction rates. Common in all environments, including where resources are scarce. Tolerant of adverse conditions but do not enter dormancy.

Cp-3: longer generation time than cp-1 and cp-2. Intermediate sensitivity to disturbances.

Cp-4: low gonad:body ratio. Long generation time. Permeable cuticle. High sensitivity to pollutants and disturbance.

Cp-5: large nematodes. Long lifespan and generation time. Low reproduction rates. Produce few large eggs. Low metabolic activity. Slow movement. Permeable cuticle. Very sensitive to pollutants and other disturbances.

Trophic groups

Plant feeders (herbivores): feeding on vascular plants; a stylet or spear is always present. Include ectoparasites, semi-endoparasites, migratory and sedentary endoparasites of vascular plants, as well as epidermal cell and root hair feeders, algal, lichen, or moss feeders.

Fungal feeders (fungivores): involves penetration of fungal hyphae by a stylet or spear.

Bacterial feeders (bacterivores): feed on any prokaryote food source.

Predators: feed on invertebrates such as protozoa, nematodes, rotifers and enchytraeids, usually with teeth or spears.

Omnivores: feed on a wide range of foods, commonly with spears.

for current thinking in nematode community ecology and for the use of soil nematode assemblages as bioindicators of soil ecosystem functions and the condition of the soil environment.

The nematode fauna provides information on two major characteristics of the soil environment and its resident communities. One is the flow of resources into the food web system, as indicated by enrichment opportunist species; the other is the trophic connectance (*sensu* Cohen, 1989) of the system, as indicated by the prevalence and abundance of higher trophic level organisms. General opportunist cp-2 nematodes are considered to be representative of organisms that persist in most soil food webs, are almost always present and are able to survive the most adverse conditions (Ferris *et al.*, 2001, 2004). The soil nematode assemblage has basal (b) (bacterivore and fungivore nematodes), enrichment (e) (resource-opportunistic nematodes) and structural (s) (omnivore and predators, more sensitive to perturbation) components (Ferris *et al.*, 2001). Based on the relative abundances of the three components, the enrichment (EI), structure (SI), basal (BI) and channel (CI) indices are calculated from the *b*, *e* and *s* characteristics of the nematode assemblage.

Abundance, biomass, carbon utilization and species diversity

The soil food web and maturity indices, as well as classical diversity indices, are all based on the proportions of nematodes in various functional guilds. They provide an indication of the relative proportions of functions that can be attributed to different guilds in the community, but not of the magnitude of such functions. The magnitude of the functions and services depend on the proportional composition of the community, but also on the biomass and physiological activity of the participating organisms. Organisms differ in their sizes, life course characteristics, activity and behaviour. It would be unreasonable to compare the amount of carbon consumed in a day by five mice and five elephants. But what if abundance was measured in terms of biomass, 1000 kg of mice compared with 1000 kg of elephants? Or, better yet, a comparison based on the metabolic footprints of the two sets of animals, taking into account the daily carbon utilized in growth, reproduction and metabolism?

The same considerations and rationale apply to the nematodes of a functional guild.

To overcome the limitations of nematode-based indicators in reflecting the magnitude of ecosystem services, Ferris (2010) proposed nematode metabolic footprints as a new family of nematode-derived indices reflecting the magnitude of some ecosystem services in which soil nematodes participated (Fig. 3.1). Nematode metabolic footprints have a production component and a respiration component. The production component is the lifetime amount of carbon partitioned into growth and egg production and the respiration component assesses carbon utilization in metabolic activity. It is calculated based on the body size, respiration rates and life-history characteristics of individual species.

Several metabolic footprints can be calculated. Bacterivore, fungivore, herbivore, predator and omnivore metabolic footprints are the metabolic footprints of nematodes in each trophic group. The enrichment footprint is the metabolic footprint of those nematodes most rapidly responsive to resource enrichment, and the structure footprint is the metabolic footprint of higher trophic levels that may have a regulatory function in the food web and which are indicative of the abundance of organisms of similar functions in non-nematode taxa. Finally, the composite metabolic footprint is calculated based on the whole nematode assemblage (Ferris, 2010).

Guidelines for Nematode Community Analyses: Tools, Interpretation and Assessment

Nematode sampling

Obtaining an adequate and representative soil sample is the first step in collecting high-quality data on nematode assemblages. As stated by Ferris *et al.* (1981), one could determine the exact number of nematodes in a field by removing all of the soil and extracting all of the nematodes. However, since cost and practicality impede such an undertaking, a compromise is to take samples and to assume that the identity and abundance of nematode populations in the samples represent the region as a whole. The greater the proportion of the area sampled, the more accurately the sampling represents the field situation.

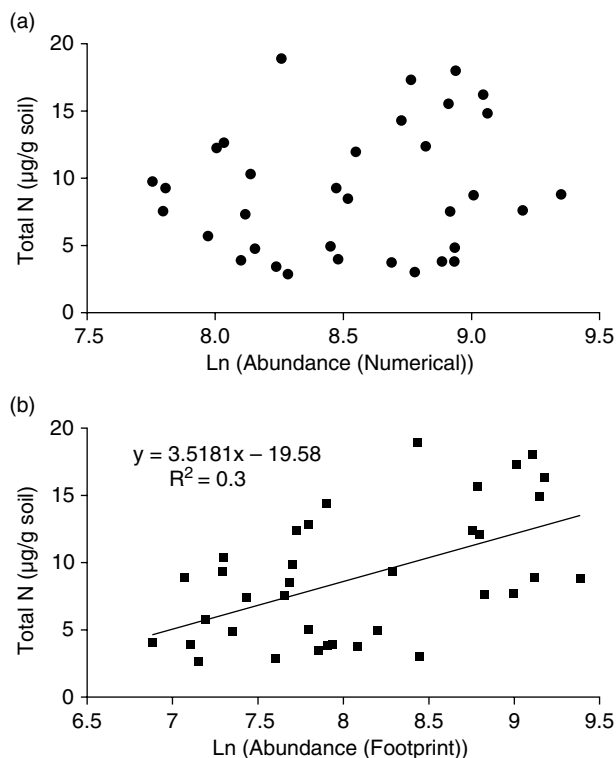


Fig. 3.1. Mineral nitrogen in field plots in relation to the abundance of bacterivore nematodes measured numerically (a) and as metabolic footprints (b). (From experimental plots reported in Ferris *et al.*, 2004.)

There are no rigid criteria for determining the number of samples in relation to the size of the field, but besides cost and time factors, other relevant considerations are:

1. Soil heterogeneity or field stratification. The area should be stratified into regions of similar characteristics of soil texture, moisture, organic matter content, salinity, differences in cropping history, irrigation practices, etc., and samples taken from each region.

2. Seasonality. Conditions and resources in the soil differ with climatic changes and crop phenology. In community ecology studies, soil sampling is typically undertaken when soil conditions are conducive to the biological activity of the target organisms. Sampling for plant parasitic nematodes is often based on the cropping schedule, e.g. before planting to determine management needs or at the end of the crop season to determine nematode abundances and the efficacy of the management.

3. Sample depth. Zones of expected biological activity in the soil should be considered. For

example, a greater abundance of plant parasitic nematodes may be associated with feeder roots, while bacterial- and fungal-feeding nematodes may be associated with surface applications of organic material. Commonly, soil nematodes are distributed from the soil surface to the rooting depth of the current plant species.

With the above criteria in mind, there are four general sampling strategies (Tan, 2005):

1. Simple random sampling. In areas of homogeneous conditions with no basis for stratification, a valid and cost-efficient approach is to collect samples at random locations throughout the area.

2. Systematic sampling. In areas of homogeneous conditions such as those in which simple random sampling might be employed, systematic samples are taken at regular intervals to ensure that all areas are equally represented by the sampling.

3. Stratified sampling. Commonly employed in heterogeneous fields. Each area of differences is considered a stratum, and all strata are sampled

separately. The number of samples collected in each stratum should be proportional to its size in relation to the whole area. Within each stratum, samples may be collected at random or systematically.

4. Compositing. In this case, the sample units, e.g. soil cores, are aggregated into a single sample. Usually, the composite sample is mixed and a representative subsample used for analysis. Although composite samples allow a greater proportion of the area to be sampled without increasing analysis and nematode identification costs, they do so at the cost of losing information on the distribution and variation in abundance of nematode populations that would be revealed by processing each sample unit individually.

Indices calculation and assessment tools

Input data

Determining the total number of nematodes in the sample is a necessary first step in the analysis. Nematodes can be counted at low magnification under a dissecting microscope, which usually takes only a few minutes per sample.

In specialized laboratories, modern technology can be used to automate nematode counting (Holladay *et al.*, 2016). Nematode identification to family, genus or species levels is commonly performed by specialists. In that case, nematodes are identified typically to genus or family level. When specialists are not available, nematodes can be identified to functional guild on the basis of feeding structures and anatomical characteristics using, for example, the features provided in Table 3.4. Finally, nematode abundance per taxonomic or functional guild is standardized to a known weight or volume of dry soil prior to further analysis.

Calculation of indices and metabolic footprints

After tabulation in standardized format, a data sheet can be uploaded into the NINJA (Nematode Joint Indicator Analysis) system. The NINJA (Sieriebriennikov *et al.*, 2014) tool is an online, freely available tool that facilitates the calculation of nematode indices. It is available at: <https://sieriebriennikov.shinyapps.io/ninja/> (accessed 10 January 2018). Following the simple NINJA instructions, the data sheet must be organized so that nematode taxa or functional

Table 3.3. Calculation of the metabolic footprint, species diversity (effective number of species) and diversity-weighted footprint for a guild of microbivore nematodes.

Genus	Mass <i>b</i>	Footprint <i>f</i>	Number <i>n</i>	Footprint $p = f \cdot n$	Calculation $r = (p/x) \cdot \ln(p/x)$
<i>Mesorhabditis</i>	0.57	0.23	132	30.69	-0.08
<i>Cruz nema</i>	10.34	2.61	396	1033.57	-0.27
<i>Diploscapter</i>	0.29	0.13	0	0.00	0.00
<i>Panagrolaimus</i>	0.64	0.25	66	16.71	-0.05
<i>Rhabditis</i>	7.50	1.83	132	241.55	-0.29
<i>Acrobeloides</i>	0.15	0.07	957	68.35	-0.14
<i>Acrobeles</i>	0.71	0.23	33	7.59	-0.03
<i>Prismatolaimus</i>	0.49	0.15	99	15.25	-0.05
<i>Aphelenchoides</i>	0.16	0.08	66	5.00	-0.02
<i>Aphelenchus</i>	0.23	0.10	1089	111.44	-0.19
				$x = \sum p$ 1530.2	${}^1D = \exp(-1 \cdot \sum r)$ 3.0
					$\theta = q \cdot s$ 4618.8

Where x = the metabolic footprint for this guild, 1D = the effective number of species (true species diversity) for the guild. In this case, we use the calculation that assigns equal weight to each species (see Ferris and Tuomisto (2015) for a more detailed explanation). θ = the diversity-weighted footprint for the guild.

Notes: For the purpose of this example, all genera are considered to be represented by a single species, so the effective number of species is synonymous with the effective number of genera. If all the genera had the same footprint, they would all be considered equally effective with regard to the function, and the effective number of species would be equal to the species richness (10 in this case).

guilds are shown in columns and cases or observations in rows. In the data sheet, the second column should indicate the variable used to group the samples (e.g. samples coming from different ecosystems, fields, crops, or from different sampling times). If no grouping variable is used, samples will be numbered consecutively. The NINJA system assigns nematode taxa to trophic groups and allows adjustments of the assignments by the user. The NINJA output contains a large information set. It includes the three main families of nematode-based indicators (maturity indices, soil food web indices and metabolic footprints), and information on the composition of the nematode assemblage in terms of trophic and cp groups.

Diversity-weighted metabolic footprints

Although measurements of species richness and diversity, or indices of diversity, of the whole nematode assemblage are interesting, they arguably do not reveal as much information about functional magnitude as the diversity of species within a functional guild (Ferris and Tuomisto, 2015). Nematode species in a functional guild differ in their size, activity, behaviour and physiological attributes. When there is diversity within a functional guild, it is more likely that the species present are able to access a greater range of substrates in a greater range of microhabitats than when the functional guild is comprised of a single species. When the nematode assemblage is assessed using taxonomic criteria to levels of species, genera and families, it is possible to calculate true species diversity, that is, the effective number of species, within a functional guild.

The calculation of the effective number of species in a functional guild is determined with the equation

$${}^qD = \exp - \sum_{i=1}^R p_i \cdot \ln(p_i) \quad (\text{Eqn 3.1})$$

when $q = 1$. Larger values of q would assign greater weight to abundant species and smaller values of q to rare species, and would require a different form of the equation. In this case we use $q = 1$, which causes each species to be weighted exactly by its proportional abundance. A summary of the steps in the calculation of true species diversity is shown in Table 3.3.

In recognition that the magnitude of an ecosystem service performed by a functional guild will be determined by the biomass and

metabolic footprint, and also by the species diversity within that guild, Ferris and Tuomisto (2015) proposed and demonstrated the use of the diversity-weighted metabolic footprint, a product of species diversity metabolic footprint within that guild. The diversity-weighted footprint (θ) is calculated as

$$\theta = ({}^qD)^b \cdot \sum_{i=1}^R A_i \quad (\text{Eqn 3.2})$$

where qD is the true species diversity, A_i is the abundance (expressed as biomass or metabolic footprints) of the R species in the functional guild of interest and b is a coefficient (0–1 range) that allows subjective adjustment of the perceived importance of species diversity. In the example in Table 3.3, we assign equal importance to species abundance and species diversity, so b has the value 1.

Precautionary caveats

Some cautions are appropriate in order to avoid bias in the index and faunal analysis calculations. Since counting, identifying and measuring all nematodes in a soil community is virtually impossible, all assessments are based on several generalizations that should be considered by the user.

Several endoparasitic nematodes, including root knot and cyst nematodes, complete their life cycles inside root tissues, where they lose their vermiform shape and become globular, increasing their biomass substantially compared to their juvenile stages. In some species, males may be present or may be in greater abundance under stressful environmental conditions. In the NINJA system, mean biomasses for genera and families are calculated from the average size of adult females. When calculating nematode biomasses and metabolic footprints, the dimensions of adult females are used for all individuals in the sample. The rationale for those calculations is that the body sizes of adult females are most frequently reported in species descriptions and that juvenile stages have the potential to reach adulthood, so that the corresponding metabolic footprint is the *potential footprint* represented by each soil sample.

The same discrepancy between juvenile and adult sizes can actually be applied to all nematode taxa. Our lack of knowledge on the real life cycle duration of most nematodes under field conditions makes the interpretation of the age

and stage structure of the nematode community difficult. Nematode generation times have been calculated in laboratory conditions for a number of species. They vary considerably among the species, from 90 h in *Caenorhabditis elegans* (Muschiol *et al.*, 2009) or ≈ 20 days in *Meloidogyne graminicola* (Fernandez *et al.*, 2014) to much longer times in predatory nematodes under field conditions (Yeates and Wardle, 1996). The alternative to using adult sizes in biomass calculations is to measure all nematode specimens in a sample, which would usually be prohibitively time-consuming. However, if differing age structures of nematode assemblages are observed, the potential for differential overestimation of nematode biomass should be recognized.

The dauer larvae of rhabditid nematodes are an alternative life stage that develops under adverse environmental conditions (Wang and Hou, 2015). Their exclusion from indices calculation has been argued; dauer larvae commonly appear under resource-limited conditions, and often represent a transient dispersal stage. Dauer larvae are usually physiologically inactive, not feeding and only minimally processing carbon. In most analyses, the exclusion of dauer larvae from index calculations is recommended, but specific applications might suggest otherwise.

A simplified faunal analysis

Herein, we present a simplified faunal analysis to allow users who do not have training in nematode identification to use evident morphological characters in categorizing nematodes into the various functional guilds (Table 3.4). Clearly, the simplified analysis does not have the resolution of, and the assessments are not directly comparable with, those derived from the system based on the identification of genera and families. However, it allows users to become familiar with the concepts and calculations involved. In this case, nematodes are assigned to feeding-habit and life-history categories (Table 3.4). Then, we use the weightings of the faunal analysis system (Ferris *et al.*, 2001) to calculate the faunal indices and the median biomass and metabolic footprints of non-plant-feeding nematodes in each cp group to calculate the enrichment and structure metabolic footprints. The median

biomass and metabolic footprint data are calculated from a large database of nematode dimensions (Ferris, 2010).

For the metabolic footprints of nematodes with different feeding habits and ecosystem function in the simplified faunal analyses, we use the median biomass and metabolic footprints of nematodes in each feeding category of the functional guild matrix (Table 3.5). The biomass and metabolic footprint of each functional guild is then calculated as the product of numerical nematode abundance in that guild and the median biomass and metabolic footprint of the guild.

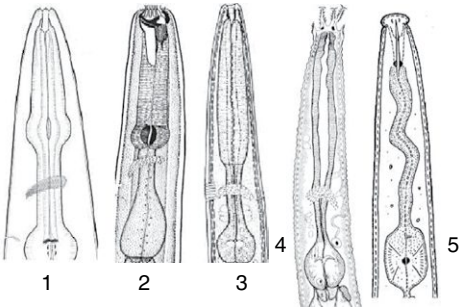
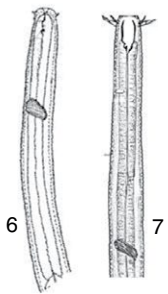
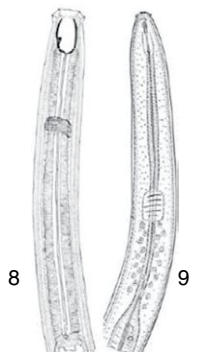
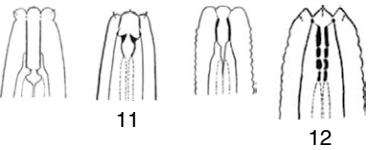
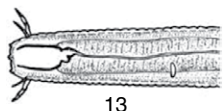
Interpretation of indices

Maturity indices

Maturity indices are indicators of the condition of the soil food web along an ecological succession. Several indices are commonly used, with minor but relevant differences among them. All maturity indices are based on the contribution of the different colonizer–persister groups to the whole nematode community, and, thus, assemblages in a less developed state along the ecological succession trajectory have lower values of the indices. Much useful information can be retrieved from the calculation of the maturity index family (Table 3.6).

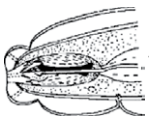
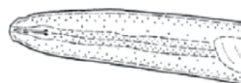
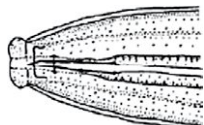
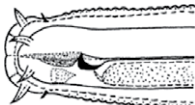
When all cp groups of free-living nematodes, including the enrichment opportunistic taxa in the cp-1 group, are included in the calculation of the maturity index (MI), low MI values indicate low maturity and a high representation of lower cp groups in the assemblage. A bloom of soil microbes occurs after mineral fertilizer is applied, organic matter is incorporated, or when conservation agriculture techniques (e.g. mulching or crop residue incorporation) are used to increase soil carbon. The bloom provides resources for bacterial-feeding cp-1 nematodes, which consequently increase in abundance. The resulting very low MI values indicate the change in the soil food web that occurs after enrichment events. When the cp-1 group is excluded from the MI calculation (MI2–5), temporal shifts in the enrichment opportunistic bacterial-feeding nematodes are ignored and the index values are attributed to longer-lasting environmental effects.

Table 3.4. A simplified chart of anatomical characteristics of nematodes in colonizer–persister categories and an outline for simplified assessments of the enrichment index, structure index, enrichment footprint and structure footprint.

	Cp-1	Cp-2	Cp-3	Cp-4	Cp-5
Anatomical characters	Valved postcorpus. Swollen metacarpus. Stoma wide.	Valved postcorpus. Metacarpus absent. Stoma narrow. Probollae.	Oesophageal cardia. Metacarpus absent. Stoma narrow or wide. Setae. Spinneret.	Oesophagus cylindrical or dorylaimoid. Oesophageal cardia. Enlarged stoma or odontostyle. Adult mean length: ± 1.7 mm	Oesophagus dorylaimoid. Odontostyle. Adult mean length: ± 2.6 mm
Anatomical characters					
Bacterivores					

Continued

Table 3.4. Continued.

	Cp-1	Cp-2	Cp-3	Cp-4	Cp-5
Fungivores					
	16	17	18	19	
Omnivores					
			20	21	22
Predators					
	23	24	25	26	27
Weighting	3.2	0.8	1.8	3.2	5.0
Median footprint	0.3051	0.0979	0.1714	0.4459	0.8761
Median species	<i>Mesorhabditis oschei</i>	<i>Aphelenchus avenae</i>	<i>Pseudoaulolaimus anchilicaudatus</i>	<i>Mylonchulus sigmaturus</i>	<i>Laimydorus tropicus</i>
Calculations	$b = 0.8 \cdot (b_2 + f_2)$ $e = (3.2 \cdot b_1 + 0.8 \cdot f_2) / b$ $s = (1.8 \cdot \Sigma 3 + 3.2 \cdot \Sigma 4 + 5.0 \cdot \Sigma 5) / b$				
Indices	$EI = 100 \cdot e / (e + b)$ $SI = 100 \cdot s / (s + b)$			Metabolic footprints $EFP = \Sigma 1 \cdot FP_1$ $SFP = (\Sigma 3 \cdot FP_3 + \Sigma 3 \cdot FP_3 + \Sigma 3 \cdot FP_3)$	

Notes: Figures from: 1. Huang *et al.*, 2014; 2, 23. Mahamood *et al.*, 2007; 3. Abolafia and Peña-Santiago, 2005; 4. Amirzadi *et al.*, 2013; 5. Esmaili *et al.*, 2016; 6, 25. Zhao, 2009; 7, 13. Tahseen *et al.*, 2006; 8, 26. Ahmad *et al.*, 2004; 9, 19. Loof and Jairajpuri, 1968; 10, 27. Mushtaq and Ahmad, 2006; 11, 12. Barri r and F lix, 2006; 14. Kiss, 2009; 15. Timm, 1969; 16. Wu *et al.*, 2001; 17. Zeidan and Geraert, 1991; 18. Nedelchev and Choleva, 1989; 20. Huang and Zhang, 2010; 21. Gagarin and Gusakov, 2014; 22.  lvarez-Ortega and Pe a-Santiago, 2010; 24. Kaisa, 2002.

Table 3.5. Simplified faunal analysis allowing a calculation of bacterivore, fungivore, herbivore and predator metabolic footprints from the median individual mass and metabolic footprints of nematode functional guilds based on life course attributes and feeding habits.

	Cp-1	Cp-2	Cp-3	Cp-4	Cp-5
Bacterivores					
Median mass	0.782	0.313	0.299	0.395	11.955
Median footprint	0.305	0.130	0.120	0.146	1.994
Number of observations	260	231	245	45	2
Median genus species	<i>Mesorhabditis oschei</i>	<i>Geomonhystera villosa</i>	<i>Prismatolaimus dolichurus</i>	<i>Amphidelus coronatus</i>	<i>Isolaimium multistriatum</i>
Fungivores					
Median mass	0.650	0.164	0.502	0.681	
Median footprint	0.263	0.079	0.179	0.222	
Number of observations	4	141	8	51	
Median genus species	<i>Tylopharynx foetida</i>	<i>Bursaphelenchus thailandae</i>	<i>Diphtherophora perplexans</i>	<i>Tylencholaimus formosus</i>	
Herbivores					
Median mass		0.133	0.489	0.554	5.426
Median footprint		0.067	0.176	0.189	1.079
Number of observations		139	462	55	113
Median genus species		<i>Coslenchus pastor</i>	<i>Merlinius gatevi</i>	<i>Trichodorus similis</i>	<i>Xiphinema index</i>
Omnivores–predators					
Median mass	0.981	0.223	1.557	2.109	3.645
Median footprint	0.367	0.100	0.432	0.531	0.793
No. observations	36	12	61	495	126
Median genus species	<i>Mononchoides leptospiculum</i>	<i>Seinura oxura</i>	<i>Tripyla setifera</i>	<i>Mylonchulus orbitus</i>	<i>Nygolaimoides borborophilus</i>
Sample calculation	Bacterivore footprint = ($\sum cp - 1n \cdot FP1 + \sum cp - 2n \cdot FP2 + \sum cp - 3n \cdot FP3 + \sum cp - 4n \cdot FP4 + \sum cp - 5n \cdot FP5$) Where <i>n</i> is the numerical abundance and FP the median footprint of bacterivores in each cp category.				

The sigma maturity index (ΣMI) accounts for all nematodes in the assemblage, including herbivore and plant parasitic species. If symptoms of plant parasitic nematode infection are detected in the field, ΣMI values might be largely driven by that community, so ΣMI values should be interpreted with some caution. To avoid such bias, ΣMI is recommended for use in natural and semi-natural systems, with low risk of large outbreaks of parasitic species.

The plant parasitic index (PPI) evaluates the complexity of the plant-feeding nematode community. It should always be interpreted in relation to specific information of the plant parasite species comprising the community. Since plant parasitic nematodes are categorized in

different cp groups, the PPI might not be a reliable indicator of expected plant damage. For example, heavy infestations of *Paratylenchus* (cp-2), *Helicotylenchus* (cp-3) or *Xiphinema* (cp-5) lead to very different values of the PPI (≈ 2 , ≈ 3 and ≈ 5 , respectively). When infestations of plant parasites are lower, the condition of the plant parasitic and herbivore nematode community may be usefully assessed by PPI values. The occurrence of sedentary endoparasitic species in PPI assessments is a concern, since only migratory juvenile stages may be encountered in soil samples, so that the abundance of the whole population is underestimated.

In all cases, the indices are based on proportions of various nematode species and functional

Table 3.6. Index name, abbreviation, range, typical values and information retrieved from maturity indices. Last column shows likely necessities of the soil system under different indication conditions.

Index	Abbreviation	Range	Values	Indicates	Likely necessity
Maturity index	MI	1–5	<2	Low soil food web maturity. Rapid bacterial-based organic matter decomposition. High fertility. Poor soil food web structure. High sensitivity to pest outbreak.	Reduce chemical fertilization. Increase complex organic matter inputs. Reduce soil physical and chemical perturbation.
			2–3.5	Intermediate soil food web maturity. Equilibrated organic matter decomposition. Medium to high fertility. Medium soil food web structure. Medium sensitivity to pest outbreak.	If poor plant growth, increase nutrient input through addition of labile and complex organic matter sources. Reduce physical perturbation.
			>3.5	High soil food web maturity. Slow organic matter decomposition, medium to low fertility. Low sensitivity to pest outbreak.	Increase labile organic matter inputs without physical disturbance.
Maturity index 2–5	MI2–5	2–5	2–3.5	Low soil food web maturity. Persistent excess of chemical fertilizers. High sensitivity to pest outbreak.	Increase complex sources of organic matter. Reduce chemical fertilization. Reduce soil physical perturbation.
			>3.5	High soil food web maturity. Low sensitivity to pest outbreak.	If poor plant growth, check for nutrient deficits.
Sigma maturity index ^a	ΣMI	1–5	<2	Low soil food web maturity. High physical or chemical perturbation present.	Reduce perturbation. Apply ecological restoration measures.
			2–3.5	Low to medium food web maturity. Intermediate perturbation present.	Reduce perturbation.
			>3.5	Mature soil food web. Good preservation condition.	Assure system conservation.
Plant parasitic index	PPI	2–5	2–3.5	Low to medium herbivore pressure.	If symptoms of infection appear, check for cp-2 and cp-3 nematodes (Criconematidae, Pratylenchidae, Hoplolaimidae, Dolichodoridae).
			>3.5	Medium to high herbivore pressure.	If symptoms of infection appear, check for cp-4 and cp-5 nematodes (Longidoridae) and/or nepovirus plant infection.

Note: ^aTo be used in natural and semi-natural systems, with low risk of plant parasitic nematode outbreak.

groups present in the sample. An assemblage of 100 nematodes may have the same index value as an assemblage of 100,000 nematodes. So, the indices are measures of potential rather than magnitudes of ecosystem functions or of the positive or negative effects on plant growth.

Soil Food Web Indices and Metabolic Footprints

The four soil food web indices (EI, CI, BI, SI) were developed to describe the overall condition of the soil food web, particularly with regard to the two main ecological services provided by soil organisms: nutrient mineralization and pest regulation or suppression (Table 3.7). The enrichment index (EI) and the channel index (CI) are indicators of active organic matter decomposition mediated by bacteria (high EI values) or by fungi (high CI values). Since bacteria primarily decompose labile organic matter and respond rapidly to fertilizer and nutrient inputs, a high EI value is an indicator of soil fertility, with abundant nutrients available for plants. However, since such labile resources are ephemeral, frequent input of nutrient sources is necessary to maintain a high level of the EI. To sustain nutrient mineralization in the longer term, recalcitrant organic matter may be incorporated into the system. Recalcitrant organic matter is primarily decomposed by fungi, so that higher values of the CI result. In such cases, if the CI is high and the EI is low, the application of some more labile materials may be necessary to meet the nutrient mineralization needs of plant growth. In agricultural systems, both decomposition channels should be coupled and active, and, in general, sources of both labile and recalcitrant organic matter are necessary at rates determined by crop growth pattern and by soil and climatic conditions.

Organic matter inputs drive other soil functions besides plant nutrient availability. For example, organic matter might suppress plant parasitic nematodes through the release of toxic metabolites during decomposition. It also provides a substrate for nematophagous fungi, many of which are facultative parasites and can be cultured without the need for nematode prey (Kerry, 2000). Also, organic amendments to soil may enhance plant resistance to biotic stress

and render the soil less conducive to pest nematode species (Oka, 2010).

The structure index and the basal index are indicators of soil food web complexity. A complex assemblage of high cp nematodes, with an abundance and diversity of omnivores and predators, is an indicator of the potential regulation of invasive and pest species. A diversity of omnivore and predator species may predate on a range of different prey, including prey of different size, behaviour and life-history strategies. Environmental conditions conducive to the presence of omnivore and predator nematodes are conducive to other organisms in similar functional guilds, including predatory mites (Sánchez-Moreno *et al.*, 2009) and other biocontrol agents (Carrascosa *et al.*, 2014). Since the structure index reflects such food web complexity, it can be considered an indicator of soil suppressiveness to opportunistic species. On the contrary, the basal index reflects the prevalence of basal nematodes, those with low cp values that survive in disturbed environments. High BI values are thus indicators of depleted soil food webs, with few enrichment opportunists and few omnivores and predators. A reduction of soil disturbance and environmental contamination is usually necessary to allow an increase in higher trophic levels and the pest regulation service that they provide. Physical and chemical perturbation (such as the use of pesticides, chemical fertilizers and tillage) have strong detrimental effects on the higher functional links of the soil food web. Conservation management strategies, including reduced tillage, maintaining soil vegetation cover, establishing crop rotation programmes, reduced use of chemical pesticides and mineral fertilizers, may improve the latitude of soil health, as indicated by the magnitude of ecosystem functions (Ferris and Tuomisto, 2015).

The graphical representation of the EI in relation to the SI is used as a diagnostic indicator of soil food web condition (Fig. 3.2; Table 3.8) (Ferris *et al.*, 2001).

Metabolic footprints are the last step in nematode-based soil food web assessment. Based on the same framework as soil food web indices, metabolic footprints provide an assessment of the magnitude of the ecological functions and ecosystem services provided by nematodes. As

Table 3.7. Index name, abbreviation, range, typical values and information retrieved from soil food web indices. Last column shows the likely necessities of the soil system under different indication conditions.

Index	Abbreviation	Range	Values	Indicates	Likely necessity
Enrichment index	EI	0–100	0–30	Poor bacterial-feeding nematode community. Few inputs of labile organic matter or fertilizers. Low participation of bacteria on nutrient mineralization. Low fertility.	Increase fertilization. Increase labile organic matter inputs.
			30–60	Medium to high inputs of labile organic matter and fertilizers. Medium to rapid organic matter decomposition. Medium to high fertility.	In the lower range (EI $\approx$ 30) increase nutrient input through addition of labile and complex organic matter source.
			60–100	Rapid decomposition, high fertility. Few complex organic matter inputs.	Increase complex organic matter inputs. Reduce chemical fertilization.
Channel index	CI	0–100	0–30	Few inputs of complex organic matter source. Low fungal participation on nutrient mineralization. Low C sequestration into complex C forms.	Increase complex sources of organic matter. Reduce chemical fertilization.
			30–60	Medium to high inputs of complex organic matter. Medium to high fungal participation in organic matter decomposition.	If concomitant with low EI, increase inputs of both labile and complex organic matter sources.
			60–100	High fungal participation in nutrient mineralization. Slow organic matter decomposition rates.	Increase inputs of labile organic matter and/or fertilizers.
Basal index	BI	0–100	0–30	Absence of soil perturbation, soil food web preserved.	Maintain low levels of soil perturbation.
			30–60	Presence of some indicators of soil perturbation. In the upper range (BI >50) soil food web is depleted.	Reduce physical and/or chemical soil perturbation.
			60–100	Soil food web dominated by indicators of perturbation. The soil food web has been seriously damaged.	Reduce physical and/or chemical soil perturbation. Restoration measures might be necessary.
Structure index	SI	0–100	0–30	Soil food web highly perturbed. Development of higher trophic links constricted. High sensitivity to pest outbreak.	Reduce soil perturbation. Assure constant C flow into top predators by applying organic matter inputs with minimum disturbance.
			30–60	Soil food web medium to highly developed. Good condition of higher trophic links, medium to high resistance to pest outbreak.	Reduce soil perturbation, if possible.
			60–100	Soil food web highly structured. Low soil sensitivity to pest outbreak, high resilience.	Maintain soil conservation.

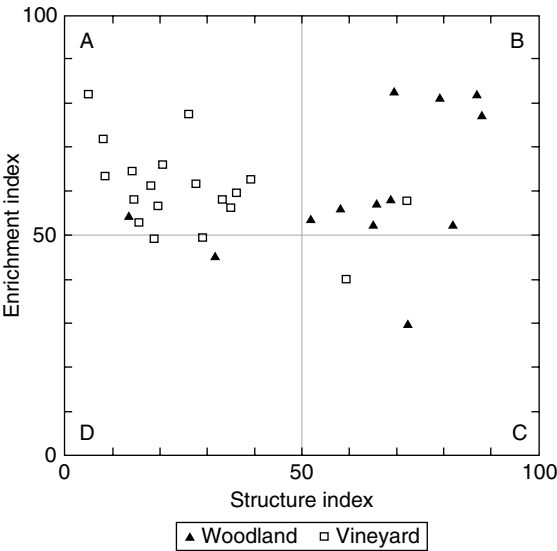


Fig. 3.2. Soil food web condition in an organic vineyard and an adjacent oak woodland in northern California (Sánchez-Moreno and Ferris, 2007). Higher EI values in the vineyard are associated with higher soil fertility, while higher SI values in the woodland are associated with higher soil suppressiveness to opportunistic or invasive species. Most samples from the woodland indicate a mature ecosystem, while those from the vineyard indicate disturbance (Table 3.8).

Table 3.8. Inferred condition of the soil food web and its environment based on weighted nematode faunal analysis. Quadrats refer to faunal ordination in the faunal profile (see Fig. 3.2). (From Ferris *et al.*, 2001.)

General diagnosis	Quadrat A	Quadrat B	Quadrat C	Quadrat D
Disturbance	High	Low to moderate	Undisturbed	Stressed
Enrichment	N-enriched	N-enriched	Moderate	Depleted
Decomposition channels	Bacterial	Balanced	Fungal	Fungal
C to N ratio	Low	Low	Moderate to high	High
Food web condition	Disturbed	Maturing	Structured	Degraded

described in their formulation (Ferris *et al.*, 2010; see the sections above on: abundance, biomass, carbon utilization and species diversity, as well as guidelines for nematode community analyses: tools, interpretation and assessment), metabolic footprints are based on the biomass and metabolic activity of the nematode assemblage within each trophic group or functional guild. In essence, the hypothesis is that the greater the total amount of carbon and energy processed by a functional guild, the greater the magnitude of ecosystem functions provided by that guild.

Although their formulation might seem complex, the functional interpretation of metabolic footprints is straightforward. Metabolic footprints of nematode trophic groups are related to the biomass and metabolic activity of nematodes in each group. Bacterivore, fungivore, herbivore, omnivore and predator footprints are indicators of the magnitude of the

function developed by each of those categories of nematodes, while the enrichment and structure metabolic footprints are indicators of the magnitude of the functions provided by enrichment and structure indicators. Plotting those seven metabolic footprints into a radar chart, which allows the observation of such multivariate data, allows us to visualize two important services provided by the soil food web (nutrient mineralization and soil suppressiveness) and an assessment of the level of herbivory to which the system is subjected (Fig. 3.3). In agricultural systems, low herbivore pressure, high mineralization service and high pest suppression are desired conditions, as represented by the cup-shaped figure (Fig. 3.3, dashed line). In an intensive agricultural system, with high organic matter inputs, high soil perturbation and intermediate to high herbivore pressure, the desired cup shape of the radar chart is lost (Fig. 3.3, solid line).

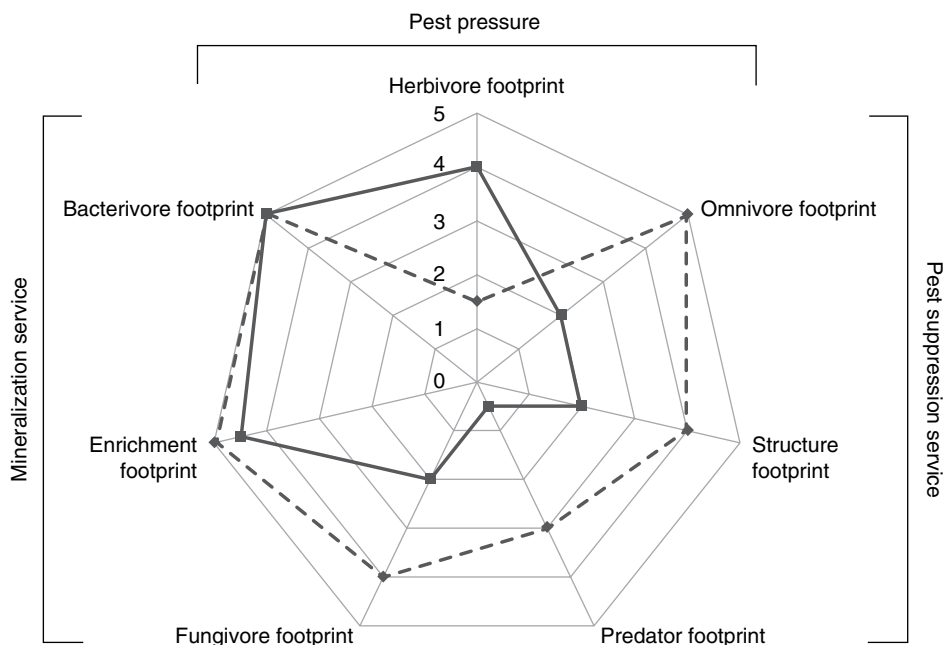


Fig. 3.3. Radar chart of nematode metabolic footprints as indicators of the magnitude of the ecosystem services provided by the nematode community and the soil food web.

Case Studies: Nematodes as Indicators of Soil Health in Agroecosystems

Soil organisms provide a large set of ecosystem services that sustain human welfare through the production of food, fibre and fuel and the maintenance of environmental quality (Tamburini *et al.*, 2016). Since the ecosystem services are derived from the complex interactions that occur within all components of the agroecosystem, they are clearly interconnected. In coffee plantations in Costa Rica, for example, coffee production, which can be limited by a number of diseases and pests, was clearly improved by farm diversification. The diversification provided an additional ecosystem service, the creation of habitats to enhance biodiversity (Allinne *et al.*, 2016). Ecosystem services provided by the soil food web are influenced by soil tillage and crop residue management, rotation systems and cover crops, the use of pesticides and fertilizers and, at a global scale, by climate change and politico-economic changes.

Tillage and crop residue management

The use of pesticides is a major agriculture-related public concern, due to the effects on the environment, on non-target organisms (such as bees, earthworms and natural enemies) and on human health. However, soil tillage may induce alterations in the soil system that are even greater than those caused by agrochemicals. Tillage, commonly used to control weeds, reduce soil compaction and to prepare the soil for seeding, disrupts soil structure and the vertical stratification of resources and organisms. It alters the spatial configuration of the microbial community, physically damages larger organisms and destroys their habitats, and in general increases loss of soil carbon through the respiration of organisms that become co-mingled with new resources. Not all soil organisms have similar responses to tillage. Although soil disruption by tillage affects all groups of soil biota (Wardle, 1995), its effects on soil health are often mediated through the incorporation of crop residues and/or soil amendments. The response of nematodes in different functional guilds depends on

their trophic habit and life-history characteristics. Consequently, tillage may result in an increase or decrease of total nematode abundance as bacterivore nematodes respond to resources provided by blooms of soil microbes.

In the absence of disruptive processes that may alter the survival of, or resources for, soil nematodes, greater species diversity is usually associated with lower soil disturbance. In a tomato–maize rotation under conventional and organic management, both nematode taxa richness and numerical abundances were reduced in field plots with greater soil disturbance. Taxa richness and nematode abundance were greater in reduced tillage systems under organic management and were less under standard tillage systems under conventional management protocols (Fig. 3.4; Sánchez-Moreno *et al.*, 2009). In a maize–soybean rotation system, Zhang *et al.* (2013) found higher nematode abundances under no tillage, followed by intermediate abundances under reduced tillage and lower nematode abundances in conventional tillage systems.

When tillage is accompanied by organic matter incorporation, opportunistic nematodes with short life cycles usually increase in abundance. Fu *et al.* (2000) found that nematode abundances increased by 270% under standard tillage, due mainly to the increase of opportunistic nematodes. The increase in abundance was accompanied by a 300% increase in soil respiration and, consequently, a 300% decrease in soil carbon. It is well established that the capacity of the soil to act as a carbon sink is reduced significantly when it is tilled (West and Post, 2002). In temperate climates, carbon loss through respiration may be as much as 98% of the carbon that has been incorporated into the soil, while only

1.8–6.5% becomes immobilized as microbial biomass and 0.01–0.12% as nematode biomass (Fu *et al.*, 2000).

The differential response of nematodes to tillage thus depends not only on their trophic group and functional guild but also on resource availability. In a humid, subtropical climate, Rahman *et al.* (2007) found that when crop stubble was removed from the field, standard tillage reduced the total abundance of free-living nematodes per kg soil from 6310 to 4266, while under stubble retention systems, standard tillage increased nematode abundance from 4169 to 7413. The increase was due mainly to the increase in enrichment opportunistic rhabditid nematodes. The stimulation effects of tillage and the incorporation of organic matter may also affect the abundance of fungivore nematodes (Liphadzi *et al.*, 2005).

As a result of the different responses of nematode species to tillage and organic matter incorporation into the soil, the structure and the function of the microfaunal food web is also modified. Since nematodes and other soil organisms at higher food web trophic levels are sensitive to soil physical disruption, the maturity (in the sense of the status with regard to ecological succession) and the structure (in the sense of the complexity of the food web) of the nematode community are both reduced. Consequently, the indices that measure such attributes (the maturity index (MI) and the structure index (SI)) are commonly reduced under standard tillage. When tillage is accompanied by organic matter incorporation into the soil, the reductions in maturity and complexity are accompanied by the positive response of opportunistic bacterial-feeding nematodes, so that the enrichment index, an

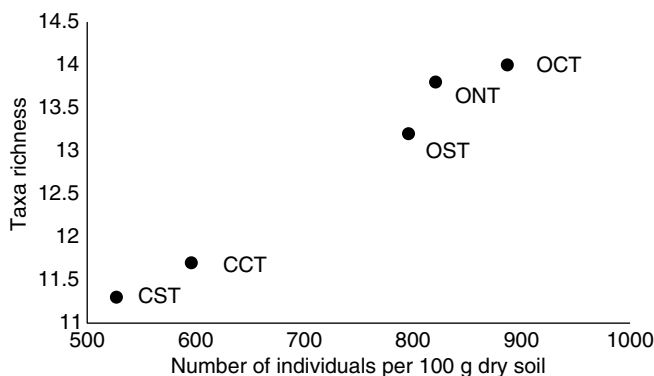


Fig. 3.4. Mean nematode abundances and taxa richness in a tomato–maize rotation system, in conventional (C) and organic (O) plots managed with standard tillage (ST), conservation tillage (CT) and no-tillage (NT). (From Sánchez-Moreno *et al.*, 2009.)

indicator of rapid organic matter decomposition mediated by bacteria, increases (Okada and Harada, 2007). At the same time, the channel index, an indicator of slower organic matter decomposition mediated by fungi, generally decreases. A reduced structure index and an increased enrichment index suggest a food web condition conducive to opportunistic pest species, typical of intensive agricultural systems.

The extent to which the changes affect soil food web functioning depends very much on tillage intensity and on the quantity and quality of the organic matter incorporated into the soil. In contrast to most faunal-based analyses, nematode metabolic footprints are indicators of the magnitude of function of each soil food web guild. Nematode biomass reacts rapidly to tillage and amendments in response both to the alteration of the structure of the three-dimensional matrix in which nematodes live and to the amount of carbon incorporated into the food web. Zhang *et al.* (2012) showed that under untilled bare soil with 0% residue retention, the nematode assemblage was well-structured, but the biomass and activity of its organisms were constrained by the limitation of resources. Under the same untilled system but with 50% of crop residue retention, the greater availability of carbon promoted a more structured microbial-feeding community. However, the microbivore biomass and microbivore activity were still constrained. With 100% residue retention, more carbon was channelled into the microbivore assemblage and diffused through the trophic exchanges of the soil food web, so that the biomass and activity of higher trophic levels were increased. Zhang *et al.* (2012) also showed that the effects on lower soil food web links of residue management under conventional tillage were greater, since carbon was incorporated more efficiently into the soil and was thus readily available to microbes, which in turn were consumed by microbial-feeding nematodes. The positive effects of increased prey availability, induced by organic matter incorporation, on large-bodied omnivores and predators are commonly obscured by the detrimental effect of the physical disruption of their habitat.

Fertilizers and amendments

Chemical fertilizers have two direct effects on the nematode assemblage. On the one hand, they

are generally toxic to soil animals, especially at the concentrations that may occur in the thin water films surrounding soil particles. Nitrogen fertilizers, including urea, $(\text{NH}_4)_2\text{SO}_4$, NaNO_2 and $\text{Ca}(\text{NO}_3)_2$ are differentially toxic to nematodes across the range of cp groups. Nematodes in higher cp groups are more sensitive than those in lower groups. For cp groups 4 and 5, the RT_{50} (the osmotic tension, measured in MPa, at which 50% of the individuals died) ranged between 0.13 and 0.30, while for cp groups 1, 2 and 3, it was between 0.25 and 2.09 (Tenuta and Ferris, 2004). On the other hand, chemical fertilizers may increase microbial resources for microbivore nematodes which, in turn, may increase in abundance. However, the increase in plant vigour stimulated by fertilizers may result in substantially greater abundances of plant parasitic nematodes. In a comparison of the effects of fertilization with NH_4NO_3 and sunn hemp (*Crotalaria juncea*) on the nematode community, Wang *et al.* (2006) found that sunn hemp increased bacterivores and fungivores, and occasionally omnivore nematodes, and suppressed plant parasitic nematodes compared to ammonium nitrate, which increased herbivores and reduced omnivore nematodes.

Fertilizers and organic matter amendments shaped the soil food web in a soybean crop, in a humid subtropical climate in Japan (Okada and Harada, 2007). Under a no-tillage system, there were few differences in the enrichment trajectories of the soil food web in response to chemical, organic and no-fertilizer treatments, probably due to low rates of nutrient incorporation in the soil in the absence of tillage. Under conventional tillage, the soil food web progressed along the enrichment trajectory from no fertilizer to organic fertilizer. Soil food web structure, besides being improved in the no-tillage system, was more complex and diverse with organic than with chemical fertilizers in both tillage systems. The soil food web was resource depleted but structured in the absence of soil disturbance and chemical perturbation, but enriched and less structured with tillage and the application of fertilizers (Fig. 3.5). The availability of nutrient inputs may play an essential role in soil food web development.

The biomass of nematodes is affected strongly by the type of nutrient inputs. At equal inputs of total N, biomasses of enrichment and structure nematode indicators were both greater

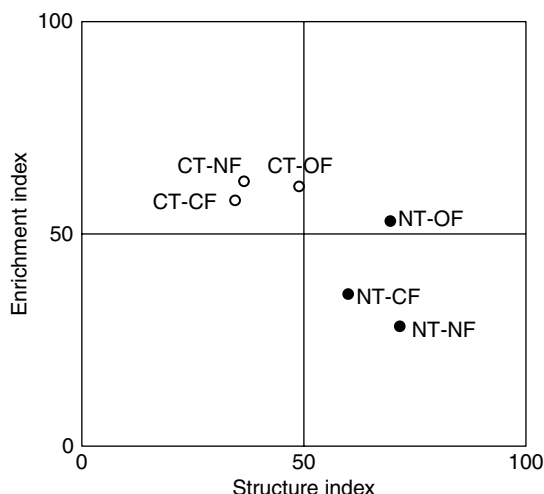


Fig. 3.5. Soil food web diagnosis of six management systems in a soybean field in Japan under conventional (CT) and no-tillage (NT) with chemical (CF), organic (OF) and no fertilizer (NF). (From Okada and Harada, 2007.)

when NPK mineral fertilizer inputs were complemented with straw or manure than when applied alone (Zhang *et al.*, 2016). Fertilizers and organic amendments not only affect microbivore and predatory nematodes; organic matter amendments may suppress plant parasitic nematodes, either through the toxic effects of metabolites released during decomposition or the stimulatory effects on the suppressive service of the soil food web. In their review, Thoden *et al.* (2011) found that organic matter did not reduce the abundance of *Meloidogyne* or *Globodera* juveniles in soil, was variable in the suppression of *Heterodera*, *Trichodorus* and *Paratrichodorus* and occasionally increased the abundance of *Pratylenchus*. In another review, Oka (2010) listed several processes by which organic matter might control plant parasitic nematodes. Among them, soil food web mediated effects were grouped under the ‘enhancement of antagonistic organisms’ category, including a number of mechanisms such as increase of predatory organisms feeding on plant parasites, the increase of nematophagous fungi and the stimulation of rhizobacteria populations that protect plant roots from parasitic nematode damage.

In banana production systems, predator–prey dynamics might be controlled bottom-up by managing soil food web resources (Ferris *et al.*, 2012). The model proposed was that the addition of organic matter into the soil initiated a trophic cascade process that resulted in increase of natural soil suppressiveness (Fig. 3.6). The decomposition of the amended organic matter supports an increase in microbes. In response to those

resources, populations levels of the ‘amplifiable prey’, that is, bacterivores and fungivores, also increase. These organisms, in turn, act as food source for omnivore and predator organisms, which results in greater predation pressure on the ‘target prey’, the plant parasitic nematodes. At the same time, facultative parasitic nematophagous fungi increase saprophytically on the organic material and enhance the pressure on the target prey. Such processes require some conditions to be met: (i) predator populations must be resource limited; (ii) soil conditions must be conducive to the survival and increase of predator nematodes and parasitic fungi; and (iii) predator nematodes, parasitic fungi, amplifiable and target prey must be co-located in a majority of patches associated with the resource subsidy and favourable conditions (Ferris *et al.*, 2012).

Organic matter inputs from plant roots, litter and other external sources increase microbial biomass that, in turn, is consumed by microbivores (*amplifiable prey*), which increase their abundances. Such prey are consumed by predatory nematodes and other predators, which proliferate and increase their predation pressure on plant parasitic nematodes (*target prey*). At the same time, the prevalence and abundance of nematophagous fungi may be stimulated by the organic amendment (modified from Ferris *et al.*, 2012).

Cover crops and crop rotation

Besides their positive effects on soil fertility and the reduction of soil erosion, cover crops have a major impact on soil biota by providing carbon

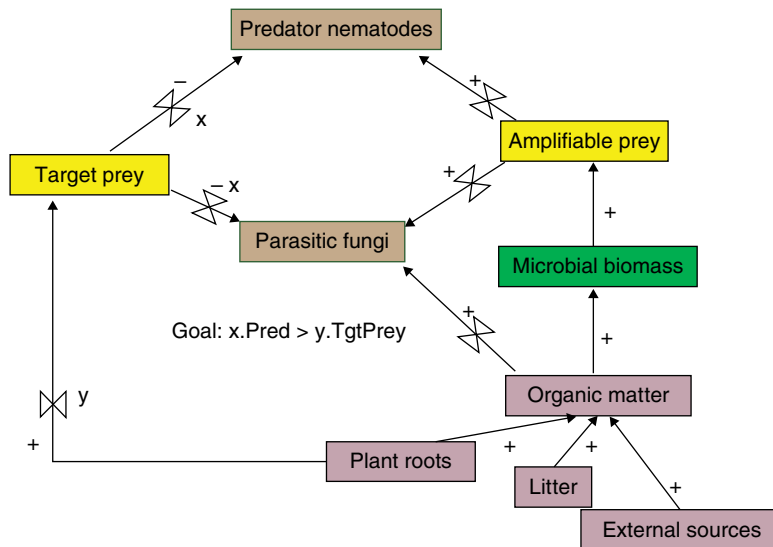


Fig. 3.6. Conceptual framework relating to organic matter inputs and soil suppressiveness developed in banana systems.

and energy to the soil food web. The type or species mixture of the cover crop determines the composition of soil organism assemblages inhabiting their rhizospheres. The quality and quantity of organic matter that they provide determine other soil food web attributes. Cover crops with low to medium carbon to nitrogen ratios induce large increases in the abundance of enrichment opportunistic nematodes and, consequently, the rate of nitrogen mineralization (DuPont *et al.*, 2009). Cover crops may also increase the abundance of organisms at higher trophic levels in the soil food web, especially where there are no constraints to such increase (Wang *et al.*, 2011).

Legumes are among the most commonly used cover crops, due to their nitrogen-fixing attributes and generally low carbon to nitrogen ratios. Used alone or in combination with grasses, legume cover crops significantly increase resources for enrichment opportunistic nematodes compared to grain cover crops or soil maintained in a bare fallow condition. Such increases result in high values of the enrichment index, which serves as an indicator of the effect of the cover crops in increasing soil fertility and nutrient availability. In a banana plantation in Martinique, legume cover crops increased the abundance of bacterivore, omnivore and root-hair-feeding

nematodes compared to non-leguminous cover crops (Djigal *et al.*, 2012). Some cover crops reduce the abundance of plant parasitic nematodes (Wang *et al.*, 2011), but others may increase it. The host status of candidate cover crops to nematodes resident in the field should always be considered in the management decision process. Cover crops and cover crop mixtures that include hosts to nematode species likely to cause damage to economic crops in the rotation should be avoided.

Pesticides

Pesticides are often considered a necessary input for safe, profitable and abundant crop yields. However, their effects on human, animal and ecosystem health, and associated regulatory limitations to their use, create pressures for the development of alternative high-yielding agricultural management strategies. There are two simple methods for determining the effects of pesticides on agroecosystem functioning. One is to compare the magnitude of the functions between organic and conventional management systems, and the other is to compare ecosystem functions of agricultural fields with those of adjacent natural systems not affected by agrochemical disturbance.

For the past several decades, plant parasitic nematode management has been accomplished effectively in many crops by the use of nematocides. Among them, soil fumigants have been a central component of many conventional production systems. However, the effects of fumigants on non-target organisms, and their environmental impacts, have led to use restrictions in many countries. Soil fumigants affect the whole nematode assemblage, and many other food web organisms. Their effects on non-parasitic nematodes may be differentially greater on higher trophic level omnivore and predator species than on bacterivores and fungivores, either due to differences in sensitivity or differences in rates of recovery from the perturbation.

Among the changes detected in soil food webs in transition from conventional to organic management may be a reduction in the abundances of plant parasites and an increase in bacterivores and fungivores (Tsiafouli *et al.*, 2007). Since some management practices may reduce the abundance of large sensitive predators and omnivores, the suppressiveness of soil to opportunistic and invasive species is often correlated with soil food web structure and the value of the structure index (Sánchez-Moreno and Ferris, 2007). In a series of experiments in disturbed and undisturbed environments, soil suppressiveness against *Meloidogyne incognita* was significantly related to the magnitude of the predator metabolic footprint (Steel and Ferris, 2016).

Under experimental conditions, the suppressiveness of soil to the parasitic nematode *M. incognita* was reduced from 91% to 61% when soil was artificially defaunated. The absence of soil organisms significantly reduced the ability of the soil food web to suppress introduced parasitic species (Sánchez-Moreno and Ferris, 2007). Pesticides thus not only suppress plant parasitic nematodes but also suppress the natural ability of the soil biota to regulate or suppress invasive and pest species. In intensively managed strawberry production systems in southern Europe, abundances of plant parasitic nematodes are extremely low, and bacterial- and fungal-feeding nematodes with low cp value dominate the soil nematode community. The soil at the edges of the strawberry fields has intermediate levels of perturbation, while those of the adjacent pine-woods are essentially undisturbed. The ability of the soil food web to suppress the introduced wax

moth larvae, *Galleria mellonella*, was progressively reduced, from high larval mortality in pinewood soils to low larval mortality in farm soils (Carrascosa *et al.*, 2014). Although such difference cannot be attributed exclusively to their use, soil fumigants surely played a relevant role in depleting the soil food of the strawberry fields.

Nematode metabolic footprints provide an overall assessment of the soil food web condition. Radar diagrams, as shown in Fig. 3.3, depicting the magnitude of seven metabolic footprints related to two soil food web services (nutrient mineralization and soil suppressiveness) and one disservice (herbivore pressure) differed among conventional and organic vineyards and olive groves growing in semi-arid conditions in central Spain (Fig. 3.7). In such systems, nutrient mineralization, assessed by the metabolic footprints of bacterivores, fungivores and enrichment indicators, and the inferred suppressive service suggested by the omnivore and structure metabolic footprints, were greater in organic olive groves than in the other systems. Vineyards, especially those under organic management, were subjected to a large herbivore nematode pressure, but still suggested more suppressive assemblages in organic than in conventional systems.

Future Directions: Molecular Identification

The necessity of taxonomic expertise to identify nematodes is often argued to be the main limitation for the use of nematode assemblages as indicators of soil condition. The evolution of new identification techniques will provide a necessary advance in soil ecology, and nematode ecology, through rapid and reliable techniques for the identification of soil organisms. Various molecular techniques have been applied to soil nematode identification, including analysis of the 18S rDNA (Griffiths *et al.*, 2006), T-RFLP and dT-RFLP (terminal- and directed terminal-restriction fragment length polymorphism) (Donn *et al.*, 2008, 2012; George and Lindo, 2015). When compared, morphological and molecular identifications are usually consistent, but resolution of molecular identifications has varied in different studies. Griffiths *et al.* (2012)

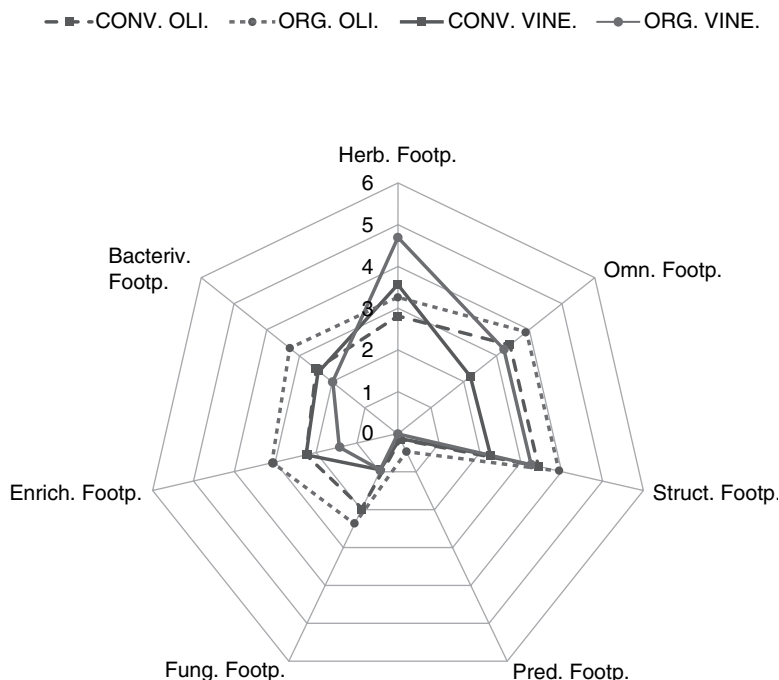


Fig. 3.7. Graphical representation on nematode metabolic herbivore (Herb.), omnivore (Omn.), structural (Struct.), predator (Pred.), fungivore (Fung.), enrichment (Enrich.) and bacterivore (Bacteriv.) footprints (Footp.) in a radar chart. Data are from organic (ORG) and conventional (CONV) vineyards (VINE) and olive (OLI) groves in central Spain (Sánchez-Moreno *et al.*, unpublished data).

were able to identify 18 nematode taxa using dT-RFLP, and observed relevant changes in total nematode abundance and the nematode channel ratio in response to soil management. A limitation of DNA-based molecular techniques is that matching sequences for specific nematode taxa may not yet be available in molecular databases (De Ley *et al.*, 2005). Porazinska *et al.* (2010) found 214 nematode species based on molecular criteria, of which only eight matched unequivocally with known species in available sequence databases. In later analyses, Porazinska *et al.*

(2012) found that up to 17% of all high-quality pyrosequencing reads were chimeric sequences coming from at least two different DNA parental molecules artificially formed during the DNA analysis. They cautioned against the probability of overestimation of biodiversity when based on techniques utilizing PCR amplification. However, and in spite of such limitations, significant advances in the molecular identification of soil organisms are occurring, and they will surely permit accurate, rapid, inexpensive identification of soil nematodes.

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4 Methods for Extraction, Processing and Detection of Plant and Soil Nematodes*

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Diagnosis of nematode damage requires methods for their extraction, handling and detection. The methods take advantage of the size, density and motility of the nematodes to separate them from plant tissue and soil particles by means of sieving, centrifugation and filtration. Different methods allow different applications, such as for diagnosis, determination of infestation levels, monitoring nematode densities and statutory testing for the presence of quarantine nematodes. Besides morphology and morphometrics, molecular techniques are increasingly used for the rapid and accurate identification of nematodes. This chapter provides details on the most common methods, while various modifications to these techniques are mostly determined by local supplies, availability of equipment and operating conditions. Further guidance, with excellent sections on methodology according to different situations, include: Thorne (1961), Ayoub (1980), Zuckerman *et al.* (1985), Southey (1986), Dropkin (1989), Hunt and De Ley (1996), Shurtleff and Averre (2000), Machado *et al.* (2010), EPPO (2013) and Coyne *et al.* (2014).

Sampling

Plants that are heavily stunted and damaged may have too small a root system to support many nematodes, and samples from nearby, less affected plants may yield more specimens. Most migratory plant parasitic nematodes are found around plant roots, and so soil samples from the rhizosphere are preferable. Usually, few nematodes occur in the top 5 cm of soil, which can be discarded from samples. Soil samples are generally taken to a depth of 15–20 cm, but 60 cm may be appropriate for nematodes affecting deep-rooted perennial and tree crops. Nematodes are not distributed uniformly in soil. Areas of nematode damage may be circular to oval or rectangular in outline; patches of poor growth may follow the rows. Sampling for stem and foliar nematodes should be from symptomatic plants. Soil samples and plant material to be examined for nematodes should be kept moist. Polythene bags are excellent containers for samples; soil and/or roots keep well in them, but whole plants are best kept separate from soil. Leaf and stems usually decompose faster than roots and should be stored in separate bags. Warm storage above

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20°C adversely affects nematodes from plants and soil, so samples should be kept cool, at around 5°C in temperate regions, 10–16°C in warmer regions of the middle latitudes and 16–18°C in the tropics and subtropics. Although it is common practice to store samples in refrigerators, low temperature (~5°C) can adversely affect the recovery of some nematodes from tropical soils (Whyte and Gowen, 1974). For more information on sampling procedures, especially sample size and sampling intensity for different crops, see Shurtleff and Averre (2000).

Fixation of Plant Tissue and Soil

In most cases, plant tissue and soil samples should be processed for nematodes within a few days after sampling. However, fixation of plant tissue and soil can be useful in preventing population changes during extended storage and avoiding quarantine restrictions applicable to live material. Roots and shoot tissue can be fixed for storage, subsequent examination or staining by adding to them preferably hot (60–70°C) formal acetic acid (FA, 4:1) or 5% formalin (2% formaldehyde solution). Alternatively, fresh material can be put directly into hot lactoglycerol; this softens tissues and is particularly helpful in the recovery of *Meloidogyne* females from roots. For soil samples, Elmiligy and De Grisse (1970) mixed hot fixative (100 ml of 40% formaldehyde + 10 ml of glycerol + 890 ml of distilled water at ~80°C) with soil samples. Nematodes in soils treated by fixation are extracted using centrifugal flotation.

Materials for Nematode Extraction

Extraction and handling of plant parasitic nematodes require mainly basic materials, which can be bought at the local market (e.g. sieves, dishes, flasks, filters, funnels and tubing) or made individually (e.g. nematode transfer pick, counting dishes, sieves and racks). Plastic or stainless steel is preferable for nematode extraction rather than brass/bronze gauze, rings or pans because metallic ions, especially copper, released into small volumes of static water can be toxic to nematodes, especially dorylaims

(Pitcher and Flegg, 1968). However, brief contact with metal sieves, as in the sieving technique, does not appear to be harmful. Stainless steel sieves are available from suppliers, but alternatives can be made using nylon gauze fixed to vinyl rings cut from plastic drainpipe of 15–20 cm in diameter.

Several methods rely on nematode mobility and their ability to pass through a filter, thus separating them from plant debris and soil particles. Cotton wool milk filters, wet-strength paper handkerchiefs and towels are suitable, as are various types of cotton cloth or muslin. Tissues containing odour or toxic substances should be strictly avoided. It is necessary to select a filter that retains as much debris as possible but with sufficiently large pores for the nematodes to migrate through. For large nematodes, such as *Longidorus* spp., a nylon gauze of about 90 µm aperture, secured to a supporting ring, will often give a clean enough extract. Various grades of lingerie material, nylon or terylene, are also suitable. Supports to hold the sample above water level can be made easily by fixing wet-strength viscose or wire mesh between two vinyl rings cut from a drainpipe. A detailed analysis of the cost-benefit ratio of extraction methods, including the advantages and limitations of each method, is given in the EPPO standard PM 7/119 nematode extraction (EPPO, 2013).

Direct Examination of Plant Material

Nematodes can usually be observed by examining small amounts of rinsed plant tissue, such as roots, leaves, stems or seeds, with a stereoscopic microscope at magnifications from 15 to 50× using transmitted and/or incident light. Examine the plant tissue in water in an open Petri dish or large watch glass, and tease it apart with strong mounted needles. Nematodes released from the tissues will float out and can be collected with a handling needle or fine pipette. Migratory endoparasites (e.g. *Aphelenchoides*, *Ditylenchus*, *Hirschmanniella*, *Pratylenchus*, *Radopholus* and *Bursaphelenchus*) emerge quickly and can be found moving about on the bottom of the dish. Sedentary endoparasitic nematodes (e.g. *Globodera*, *Heterodera*, *Meloidogyne* and *Nacobus*) may be seen attached to the surface of roots or in dissected tissue. Semi-endoparasites

(e.g. *Rotylenchulus* and *Tylenchulus*) and firmly attached ectoparasites can be seen attached to the surface of the roots. Since nematodes tend to migrate from damaged tissue, it is often worthwhile to re-examine the sample after a few hours.

To recover females of root knot nematodes (*Meloidogyne* spp.) from roots, carefully tease away the tissue with forceps and a fine needle to release the head and neck; avoid puncturing the body. Dissection and storage in 0.9% NaCl helps to avoid the osmotic effect of water, which tends to cause females to burst.

Staining of nematodes in plant tissue

Since nematodes are translucent and difficult to see in plant tissues, staining helps to visualize them. Plant material needs to be rinsed free of soil, and thick material sliced thinly before staining. Detection of *Meloidogyne* females can be facilitated by staining the roots in 0.4% cochenilla red food stain for 15–20 min, rinsing and examining them in water; the gelatinous matrix of the egg sac is stained red (Thies *et al.*, 2002).

When staining specimens within leaves, stems and roots, the plant tissue needs first to be cleared in diluted sodium hypochlorite bleach (5.25% NaOCl or Clorox) for about 4 min. Prior assessment is needed to determine a suitable concentration and incubation time for the target tissue, e.g. thin, soft tomato roots clear quickly, but tough, woody coffee roots are difficult to clear. Thoroughly rinse the roots on a 100 µm aperture sieve to remove all traces of the bleach, which inhibits staining by acid fuchsin. Transfer the plant material into a glass vial and cover it with the acid fuchsin solution (3.5 g acid fuchsin, 250 ml acetic acid, 750 ml water, diluted 1:40 with water before use). Boil the solution for a few seconds in the case of seedlings and for up to 30 s for mature tissue in a microwave oven or on a hot plate in a ventilated area, to avoid the vapour of acetic acid. Permit plant tissue to cool in the stain before transferring to a sieve (100 µm aperture) to rinse off excess stain under running tap water. In case of nematode quantification, be aware that the boiling procedure may release nematode stages from the root tissue that appear in the staining solution. If destaining with tap water proves insufficient, transfer the tissue to a solution of glycerol and distilled water,

in equal volumes, acidified with a few drops of acetic acid. Depending on the thickness of the material, differentiation may take from several hours to 2–3 days, but the stained nematodes should be seen eventually in largely unstained tissue. Alternatively, plant tissue can be stained in acidified lactoglycerol plus 0.05% acid fuchsin or 0.05% methyl blue stain for a few minutes (Bridge *et al.*, 1982), or in 12.5% (v/v) McCormick Schilling red food colour for 20 min (Thies *et al.*, 2002).

Extraction from Plant Material

The most commonly used methods for the separation of nematodes from plant material rely on nematode activity (e.g. modified Baermann funnel technique), which are therefore not suitable for extracting slow-moving (e.g. *Criconeimoides*, *Hemicyclophora* and *Xiphinema*) or sedentary nematodes (e.g. *Globodera*, *Heterodera*, *Meloidogyne*, *Rotylenchulus* and *Tylenchulus*), although juveniles and males of such forms will usually be recovered. For the latter, maceration–filtration or the mistifier technique are more suitable. Comparing the efficiency of these three techniques to extract *Pratylenchus zeae* and *Hirschmaniella oryzae* from rice roots, Prot *et al.* (1993), found the maceration–filtration or mistifier techniques most efficient. Other, less often used methods include the incubation technique (Young, 1954; West, 1957). Nematode extraction from bulky plant substrates, such as bulbs, corms or enlarged storage roots, can present difficulties. In such cases, the plant tissue can be peeled and used for nematode extraction to provide reliable data (McSorley *et al.*, 1999).

Baermann funnel technique

The Baermann funnel technique in its original form should no longer be used, as nematode recovery is less than 20% of that of other methods (Oostenbrink, 1970), mainly because of anaerobic conditions due to bacterial decay of the submerged organic matter and lack of oxygen at the base of the funnel stem. However, this technique has been modified in several ways to become a standard method for extraction of nematodes from plant tissue and soil.

Modifications of the Baermann funnel are used widely to extract active adult and juvenile nematodes (e.g. *Anguina*, *Aphelenchoides*, *Ditylenchus*, *Hirschmaniella*, *Pratylenchus* and *Radopholus*). Examples of modified Baermann techniques are illustrated in Fig. 4.1a–e. The funnel technique uses a supporting mesh (see the section on materials for nematode extraction) to hold the plant tissue partly submerged in water, to avoid anaerobic decomposition (Fig. 4.1b). A milk filter or paper tissue is placed on the support and the chopped plant material placed upon it. Fill the funnel with tap water and set the sieve in the funnel to submerge the filter partly but not completely. After 24–48 h, collect the nematode suspension as described above.

Using a shallow tray, dish or bowl (Whitehead and Hemming, 1965; Rodríguez-Kábana and Pope, 1981) instead of a funnel further improves oxygenation and reduces the number of nematodes remaining on the funnel wall (Fig. 4.1c and d). As above, a milk filter or paper tissue is placed on a support and the chopped plant material placed on it. A circle of muslin or paper tissue placed on top of the material will keep it moist and prevent it from floating. The support, with the sample material, is placed in a tray filled with tap water. Glass rods or small feet attached to the sieve ring provide a space of about 5 mm between the base of the sieve and the collecting tray. The material should be almost submerged. When adding water, do not pour water over the sample to avoid washing debris through the filter. Avoid too large sample sizes; split the sample or use larger trays of 20–30 cm in diameter instead (Fig. 4.1e). After 24–48 h, gently remove the support with the sample and transfer the suspension to a beaker. The sample can be re-immersed in fresh tap water for further extraction of nematodes. Oxygenation, hence nematode extraction, can be improved by wetting the roots with tap water containing 1–3% H_2O_2 (Tarjan, 1967). H_2O_2 helps in extracting migratory endoparasites from fleshy roots (e.g. banana), especially where high temperatures reduce oxygenation.

Mistifier technique (Seinhorst, 1950)

Nematodes recovered using this method are more active than by the previous methods because oxygenation is better, and sap and

decomposition products from the material, which inactivate nematodes, are washed away. A fine mist of water is sprayed over the plant material using about 4.5 l/h. Most systems use an intermittent spray of ~1 min every 10 min. Oil burner nozzles or gas jets can sometimes be adapted, and a water pressure of ~2.8 kg/cm² is usually required to give a suitable mist. Plant material is chopped finely to ~3–4 mm long and placed on a milk filter or tissue supported on a mesh set in a funnel or dish for the modified Baermann technique (Fig. 4.2). Optimum sample size depends on sieve diameter and water flow rate; increasing sample size can decrease the efficacy of extraction (De Waele *et al.*, 1987). Nematodes collected in the funnel tube can be released into a beaker. Compared with the modified Baermann techniques, plant tissue will decompose much more slowly, thus allowing prolonged extraction times of up to 3 weeks (e.g. freshly hatched *Meloidogyne* juveniles). Several funnels can be arranged simultaneously on a rack, with one or two nozzles supplying them all. The whole apparatus can be established on the bench if enclosed with a polythene cover and left to stand on a drainage tray. For a more elaborate apparatus using collection trays instead of beakers, see Southey (1986).

Maceration techniques

Maceration is used for extracting active nematodes as well as immobile stages of sedentary nematodes from bulbs, cloves, corms, storage roots, crowns, leaves and small plants. The plant material is chopped into ~1 cm lengths and then macerated in about 100 ml of water in an electric blender. The maceration time required depends on the type of mixer used and on the type and thickness of plant material. Maceration needs to be sufficient to enable easy egress of nematodes from the tissues but not render them immobile. For the extraction of eggs (e.g. *Meloidogyne* spp.) from root tissue, the sodium hypochlorite (NaOCl) technique described by Hussey and Barker (1973) is recommended. Maceration methods in general are often quicker and more efficient than those described previously. However, the maceration action may release toxic substances from the plant material that can kill or immobilize nematodes. Toxic substances can

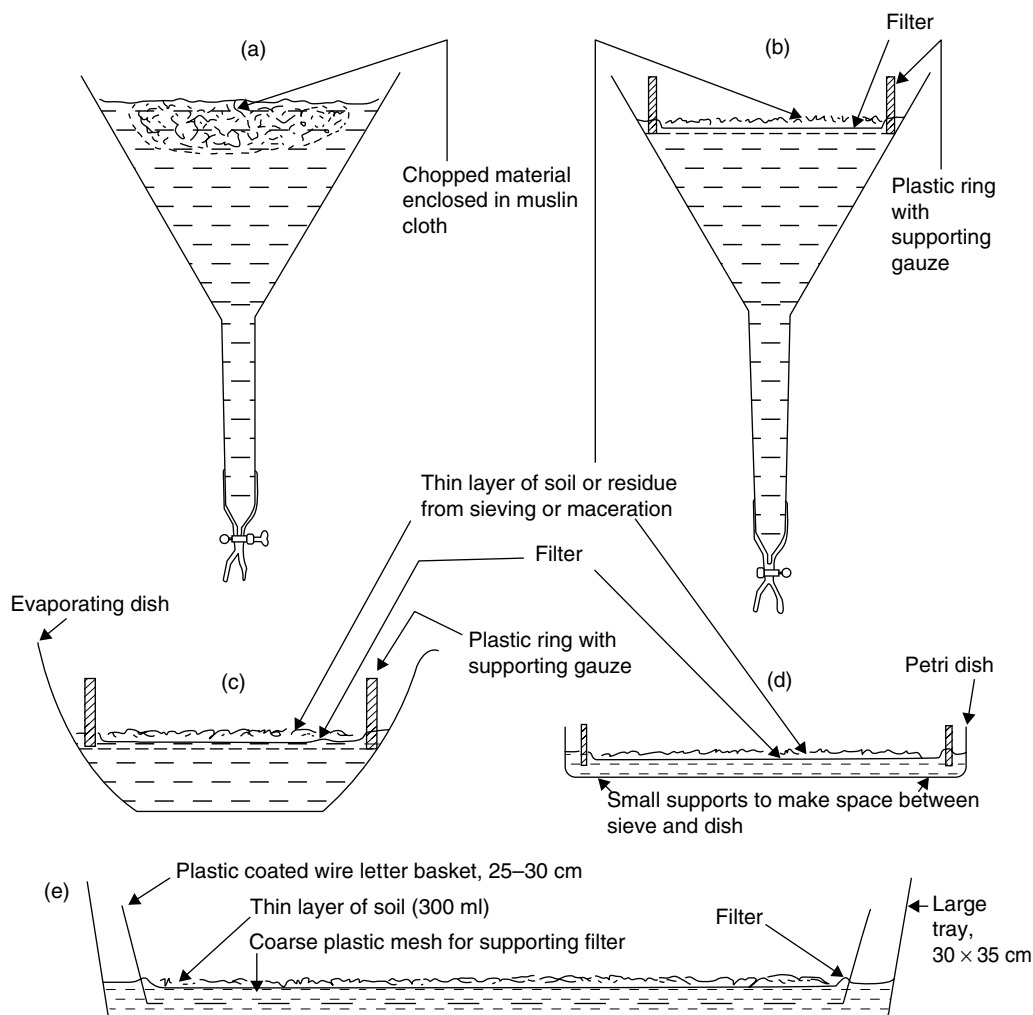


Fig. 4.1. Baermann funnel and modifications for extraction of active nematodes from plant material. (a) Original Baermann funnel technique with plant sample submerged in water. (b) Modification of placing the chopped plant material on a supporting mesh placed in a funnel. (c) Modification of using a bowl instead of a funnel. (d) Modification of using a dish instead of a funnel. (e) Modification of using a tray for large sample sizes. The filter is a cotton wool milk filter, wet-strength facial tissue, coarse cotton cloth, or fine woven nylon or terylene cloth. Plastic rings are cut from perspex, polythene or vinyl tubes. The supporting gauze is a muslin or nylon cloth held with an elastic band, or a coarse plastic mesh stuck or fused to the edge of the ring.

be partially removed and extraction efficacy improved by pouring the macerated debris and water through the filter on the Baermann dish, removing the water in the dish and refilling the dish with tap water. Plant debris hindering nematode observation can be cleaned by the modified Baermann technique (see above) or centrifugal flotation.

For centrifugal flotation (Coolen and D'Herde, 1972; Coolen, 1979), the macerated plant sample is poured on to a 1200 μm aperture sieve resting in a funnel standing in a 500 ml centrifuge tube. The residue on the sieve is washed carefully with a spray before it is discarded. A 5 ml aliquot of kaolin powder is added to the extract in the centrifuge tube and the contents thoroughly

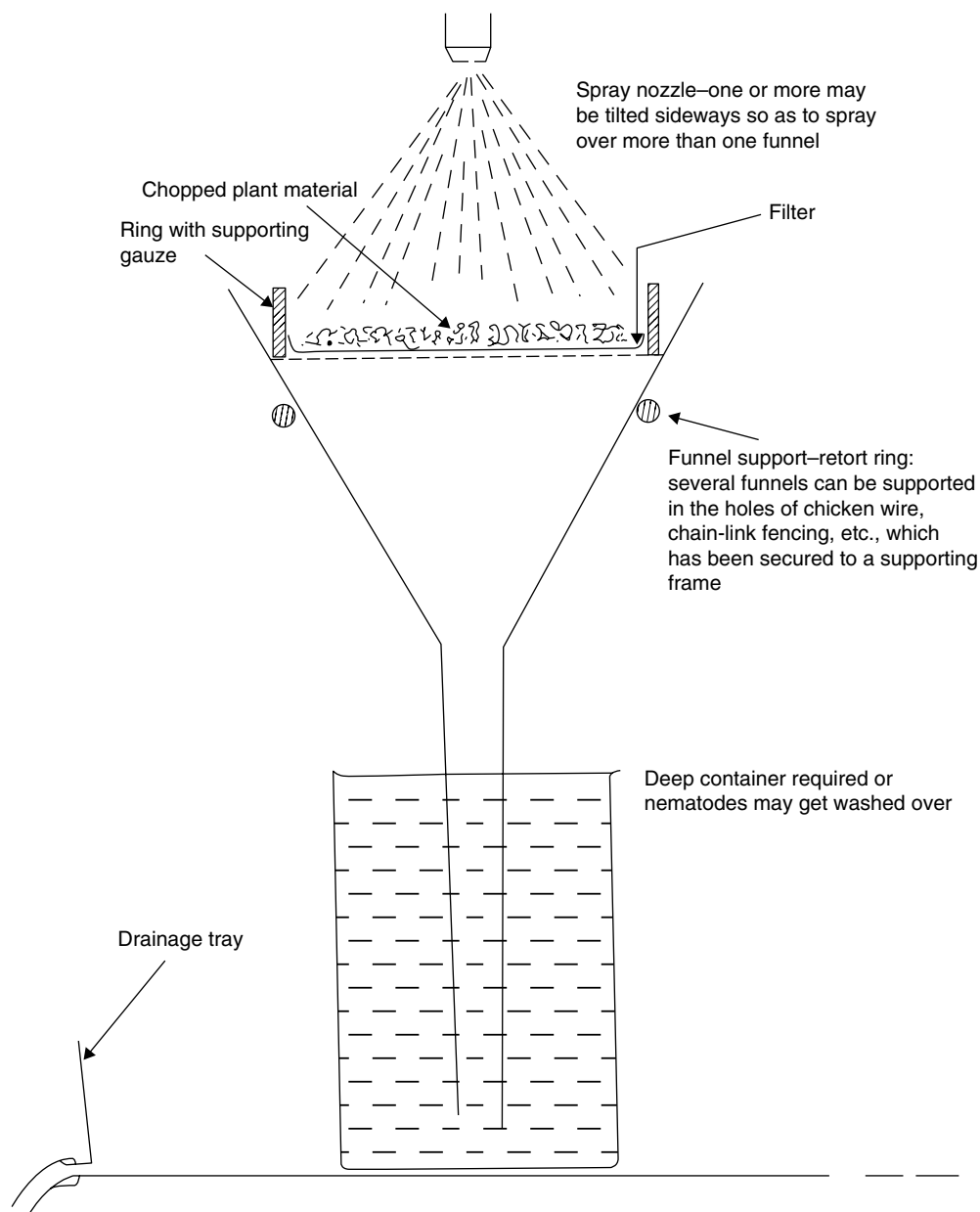


Fig. 4.2. Mist extraction of active nematodes from chopped plant material. The apparatus may be covered with plastic sheeting to prevent spread of the spray.

mixed with a Vibromixer. Tubes are balanced and centrifuged for 4 min at 1500 g; the supernatant is poured off and the residue resuspended in sucrose, ZnSO_4 or MgSO_4 solution of specific gravity 1.18 and mixed with a Vibromixer or manually for at least 30 s. Tubes are balanced with the appropriate solution and centrifuged for 4 min at

1500 g. The supernatant is then poured through a 20 μm aperture sieve, and the nematodes and eggs collected in a beaker. De Waele *et al.* (1987) found that the efficiency of extraction of *Pratylenchus* from maize roots decreased with an increase in sample size, and so the root mass treated should be standardized for comparative studies.

Extraction of *Bursaphelenchus* from stem tissue

For stem tissue, chop and macerate in a blender for 2 min and then transfer contents to a 2 l conical flask filled with water and allow to stand for 30 min to permit the nematodes to emerge; shake the flask and invert with the neck in a vessel of water and allow the suspension to settle for 30 min. The contents of the lower vessel are discarded and the flask contents are sieved four times through a 63 µm aperture sieve; the residue is washed off each time and collected in a beaker (after Fenwick, 1963). A comprehensive discussion of this method can be found in Ayoub (1980).

Extraction from Soil

Before starting nematode extraction, pass the soil through a coarse sieve of ~1–2 mm to break up clumps and remove stones, roots and plant debris. Then, mix the soil thoroughly and remove a subsample using a beaker of known volume. A 100 ml soil volume is commonly used. Nematode extraction from soil requires techniques different from plant tissue, except for the modified Baermann technique. However, this technique is inefficient in recovering large, slow-moving nematodes (e.g. *Longidorus* and *Xiphinema*) or nematodes with cuticular appendages (e.g. Criconematids). These are best extracted using sieving or elutriation techniques. Sieving or sieving plus filtering are quick methods for assessing all types of active, inactive and dead nematodes in soil, but they are not very quantitative as they are subject to much operator error. Elutriation techniques are very versatile methods capable of extracting wet cysts and vermiform nematodes from soil or root knot females from root debris, providing the appropriate sized sieves and the correct flow rate of water are used. Flotation techniques give the most efficient and quickest extraction of active and sedentary nematodes from soil. Ideally, large centrifuge tubes (300–1000 ml) are preferable, but smaller tubes can be used, especially when used in conjunction with a sieving technique. Other, less frequently used techniques include the Seinhorst two-flask technique, which is a simple method giving a more efficient and cleaner extract than direct sieving (Seinhorst, 1955). A combination of techniques

can improve accuracy of the assessment, as noted by Demeure and Netscher (1973) for *Meloidogyne* in a sandy clay soil.

Comparing the different techniques, Yen *et al.* (1998) found higher recovery rates of *Meloidogyne incognita*, *Pratylenchus coffeae*, *Aphelenchoides besseyi* and free-living nematodes when using the centrifugal flotation method and flotation–sieving technique than the modified Baermann funnel method. Comparing the modified Baermann technique with flotation–sieving, Rodríguez-Kábana and Pope (1981) extracted higher numbers of *Pratylenchus*, *Meloidogyne* and *Heterodera* with the modified Baermann method, but *Helicotylenchus* and *Hoplolaimus* were higher for the flotation–sieving method. Nematode recovery, especially of endoparasitic specimens (e.g. *Meloidogyne* and *Pratylenchus*), can be improved by incubating the soil sample at room temperature for 3–4 weeks prior to extraction. Further information on the advantages and disadvantages of the various techniques is given in the EPPO standard PM7/119 on nematode extraction (EPPO, 2013).

Modified Baermann technique (Whitehead and Hemming, 1965)

The modified Baermann technique requires little labour and uses simple equipment. For soil samples up to 100 ml, flowerpot dishes or plastic bowls of 10 cm in diameter can be used. For handling larger samples, the Baermann tray or dish technique is generally preferred over the Baermann funnel technique. A support to hold the soil above water level is made from a plastic sieve or wire basket. Cotton wool milk filter or paper tissue is laid on the support. The support is held in a collecting tray (e.g. plastic dish or bowl, greenhouse tray). Up to 100 ml soil is spread thinly over the filter in the basket, which should not exceed 5 mm as extraction efficacy will decline rapidly with increasing thickness of the soil layer. Water should be added carefully down the inside edge of the collecting tray until the soil becomes wet (Fig. 4.1e). To obtain a clean extract, it is important not to move the tray once the water has been added. Space can be saved by making a simple rack to hold the trays, and evaporation can be lessened by covering with polythene sheeting. Most nematodes will have collected on the bottom of the tray after 24–48 h,

but root knot juveniles from egg masses, or some endoparasites from root fragments, may take several days to emerge. The support with the soil is then removed slowly and carefully, and the nematode suspension from the tray beneath can be concentrated by pouring into a 100 ml measuring cylinder and leaving to settle for 4 h or more, when the supernatant water can be syphoned off. Alternatively, the suspension can be concentrated quickly by passing it through a 20 μm sieve, washing the nematodes off the sieve and collecting them in a small tube/vial.

Sieving technique (Cobb, 1918)

The sieving technique is also known as the 'bucket-sieving' method. Although crude, it is widely used as it enables the extraction of large numbers of both active and inactive nematodes in a relatively short time. Equipment required includes two plastic buckets (5 l), sieves of 15–20 cm diameter made with wire mesh (preferably stainless steel) of an aperture size of 2 mm, 710, 250, 125, 90, 63, 45 and 25 μm , respectively, and tall 100 ml measuring cylinders for the residue from the sieves.

Usually, only three or four of the set of sieves will be used for a particular sample, with the sieves selected to match the size of nematode it is hoped to extract, and to suit the type of soil involved. In general, sieve openings should be no greater than one-tenth of the nematode length. Most adults of large nematodes (e.g. *Anguina*, *Belonolaimus*, *Hirschmanniella*, *Longidorus* and *Xiphinema*) are caught on a 250 μm aperture sieve, adults of average-sized nematodes (e.g. *Aphelenchoides*, *Ditylenchus* and *Hemicycliophora*) on a 90 μm aperture sieve, and many juveniles and small adults (e.g. *Criconeimoides*, *Paratrichodorus*, *Paratylenchus*, *Pratylenchus* and *Radopholus*) on a 63 μm aperture. A 45 μm , or even 25 μm , aperture sieve is used to recover small juveniles (e.g. *Meloidogyne*, *Heterodera* and most others). Use sieves singly, never stack them and never attempt to work a sample through them all simultaneously, as this may reduce the efficiency of recovery. Fine sieves are easily clogged, but this can partially be avoided by pouring the suspension on a sieve inclined at an angle of about 30° to the horizontal; however, the number of nematodes caught on the sieve will also be reduced

(Araya *et al.*, 1998). Sonicate sieves for cleaning. The method is as follows:

1. Place a known volume of soil (100–500 ml) in bucket I and fill with about 1–4 l of water. Dry soils should be soaked for a few hours. The mixture is stirred to free nematodes from the soil and suspend them in the water. Flocculating agents, such as Separan NP10 (12.5 $\mu\text{g}/\text{ml}$), can be used to help to break up soil aggregates in heavy clay soils.

2. Allow the mixture to settle for 30–60 s and decant over a 2 mm aperture sieve into bucket II. Avoid pouring the sediment. Add less water to the sediment in bucket I and repeat this step 2–3 times to increase nematode recovery. Any sediment left in bucket I is then discarded and bucket I washed out. The sieve is rinsed over bucket II. The residue on this sieve may contain very large nematodes, but usually it can be discarded safely.

3. The contents of bucket II are stirred, allowed to settle for about 10 s and then poured through a 710 μm aperture sieve into the clean bucket I, leaving behind heavy soil particles to which more water is added and the process repeated, if desired. The sieve over bucket I is rinsed. The residue on this sieve may contain only a few large nematodes, but this often depends on how much debris is present. To collect the residue, hold the sieve over bucket I at a steep angle (35–45°) and direct a gentle stream of water on to its upper side to wash the nematodes to the bottom edge of the sieve. Small nematodes and eggs will be washed through the sieve into bucket I and recovered later. Transfer the nematodes on the sieve into a 250 ml beaker using a gentle stream of water, leaving behind any heavy particles.

4. Bucket II is cleaned and the process repeated using 250, 125 and 90 μm aperture sieves and collecting the residues, as described above. The residues of each sieve can be pooled in one 100 ml measuring cylinder, or kept separate in different measuring cylinders. The contents of the collecting measuring cylinders are allowed to settle for 3–4 h and the supernatant liquid decanted carefully or syphoned off, leaving about 20 ml in the bottom. The suspension can be transferred to a viewing dish and examined.

Some shorten the procedure by transferring the soil suspension directly through a 1–2 mm aperture sieve to remove very coarse material,

followed by a 45 μm aperture sieve to collect the nematodes. This procedure is less suitable for larger sample sizes (>250 ml) and heavy soil, due to clogging of the fine sieve. Although this technique is less laborious, nematode losses may be higher. If the suspension still contains a significant amount of debris, further processing by centrifugal flotation or modified Baermann techniques can result in an almost clean nematode suspension. However, sluggish and inactive nematodes can be lost (e.g. *Longidorus/Xiphinema*).

Elutriation techniques

Elutriation techniques extract nematodes from soil samples of 100–1000 ml by using an up-current of water to separate them from soil particles and hold them in suspension. They give a cleaner extraction than that obtained by direct sieving; however, further cleaning by the modified Baermann technique or centrifugal flotation might be required. Flow rates can be adjusted readily to suit soil type and the size of nematode to be extracted. Of the models that have been developed (Seinhorst, 1956; Tarjan *et al.*, 1956; Oostenbrink, 1960), the No III model of Oostenbrink is often used because it is robust and easily operated and cleaned. Oostenbrink (1960), Southey (1986) or EPPO (2013) should be consulted for details. Winfield *et al.* (1987) described a column elutriator for extracting nematodes and other small invertebrates, referred to as a Wye Washer. This equipment was shown to achieve extraction rates equal to or better than existing techniques; however, the water use and price are high. Another alternative is the fluidizing column (Trudgill *et al.*, 1973), representing a simple, robust and versatile elutriator.

Centrifugal flotation techniques

Nematodes can be extracted from soil and organic debris by floating them out in a solution of specific gravity greater than their own. As the method does not rely on the mobility of nematodes, it is extremely useful for extracting sluggish forms, such as criconematids, as well as dead, moulting or fixed nematodes and eggs. Centrifugal flotation is generally a more efficient

nematode extraction method than the Baermann, sieving or elutriation techniques. Flotation is often used to clean extracts obtained by sieving or elutriation, but can also be applied directly to soil samples. Solutions of sucrose, MgSO_4 or ZnSO_4 can be used. Sugar is the most used solute because it is cheap; however, Rodríguez-Kábana and King (1975) found that blackstrap molasses was even cheaper and, because of higher viscosity, more effective than sucrose for extracting nematodes. MgSO_4 does not have the stickiness of sugar but can be reused, and ZnSO_4 has fewer osmotic effects but is more acid and toxic. Other manufactured solutes (Ludox, Ficoll and Percoll) have advantages over MgSO_4 and ZnSO_4 but are more expensive (Viglierchio and Yamashita, 1983; Bloemers and Hodda, 1995). To reduce the osmotic stress by the solutes, nematodes should be rinsed with water as soon as possible to aid their recovery. A solution with a specific gravity of about 1.18 (673 g of sugar dissolved in water and made up to 1 l) is suitable for most nematodes; however, a more dense solution of specific gravity 1.25 (1210 g of sugar dissolved in water and made up to 1 l) is required for very long nematodes, such as *Longidorus* and *Xiphinema*. The specific gravity of a solution should be checked just prior to its use, as changes in temperature and microbial activity can cause a considerable decrease in concentration. The suspensions recovered are caught on a sieve of 20 μm aperture and used for direct counting.

For centrifugal flotation, a soil sample of 100–250 ml is placed in a 800–1000 ml centrifuge tube and water added up to 2 cm from the tube brim. Kermarrec and Bergé (1971) recommend the addition of a tablespoon of kaolin to aid sedimentation and to give a more compact surface to the sediment pellet. The contents are mixed thoroughly using a Vibromixer or mechanical device. The tubes are balanced by adding water and centrifuged at about 1800 g for 4 min. The supernatant containing organic debris is discarded and the tube almost filled with the suspending solution (specific gravity 1.18) and stirred mechanically or with a Vibromixer to re-suspend the pellet containing the nematodes. Tubes are balanced by adding more solution and centrifuged again at 1800 g for 4 min. The supernatant is poured through a sieve of 53 μm aperture or less (to avoid loss of smaller nematodes), rinsed quickly with tap water and collected in a

beaker or counting dish. The relative centrifugal force represents the force on particles due to gravity: $g = 0.00001118 \times \text{radius of centrifuge arm to tip of tube in cm} \times (\text{speed in r.p.m.})^2$. Flocculating agents, such as Separan NP10 (12.5 µg/ml), might be used to help break up soil aggregates in heavy clay soils. Large soil samples of 500–2000 ml can be processed by first applying the sieving technique followed by centrifugal flotation.

Extraction of heteroderid cysts from dry soils

The saccate dead females, ‘cysts’, containing eggs of heteroderid nematodes differ from other nematode stages in size, shape and weight. Different methods have been developed for extracting cysts from dry soil (e.g. Fenwick can, Schuiling centrifuge) and for extracting from wet or dry soil (e.g. Seinhorst elutriator, centrifugal flotation, Wye Washer). Cysts from dried soil contain air bubbles and float in water. To extract those cysts, a 100–1000 ml sample of the dried soil is placed in a plastic bucket, made up to about 2–5 l with water and thoroughly stirred with a strong stream of water or manually. Allow the coarse material to sediment for 1–3 min. Any cysts present will float to the surface with other organic debris. Decant through a 2 mm aperture sieve over a 250 µm aperture sieve (a 100 µm aperture sieve may be needed to catch small cyst nematodes, such as *Heterodera trifolii*). Repeat the process 2–3 times if necessary. Discard the residue on the 2 mm aperture sieve and collect the cysts on the 250 µm aperture sieve for further examination. Alternatively, the float can be poured on to a filter paper in a funnel, the water drained off and the paper examined for cysts, most of which will occur along the ‘tidemark’ left at the upper water level (Shepherd, 1986). Methods for extracting cysts from moist soil rely on elutriation that keeps the cysts afloat in the suspension, or on centrifugal flotation using a solution with a higher density than their own (e.g. 1.25). Based on Riggs *et al.* (1997), sieving was more efficient than elutriation for extracting cysts. If cysts are to be used further as inoculum in biotests, note that the contents of *Globodera*, but not *Heterodera*, cysts will survive desiccation. See EPPO (2013) for further details

on these methods and their advantages and disadvantages.

Storage

Many nematodes remain in good condition for several days when stored in shallow, fresh tap water at about 5–10°C. Contaminating bacteria can be suppressed by adding three drops of 5% streptomycin sulfate solution per 5 ml of suspension. Tropical nematodes needed for live cultures or for experimental use should be stored at room temperature and aerated with an aquarium pump. For long-term storage (e.g. germplasm collection, maintenance of genetic lines, reference material or inoculum), nematodes can be stored in liquid nitrogen. Cryopreservation has been shown to work for several nematodes (Irdani *et al.*, 2011). For *Pratylenchus thornei*, the survival rate was 76% when nematodes were pre-treated in 14–17% glycerol for 5 days before storage in liquid nitrogen (Galway and Curran, 1995). Thawed nematodes were able to reproduce and infect carrot disc cultures. Similar survival rates were achieved by van der Beek *et al.* (1996) for *Meloidogyne hapla* and *Meloidogyne chitwoodi* in liquid nitrogen after pre-treatment in 10% ethanediol for 2 h at room temperature and 40% ethanediol for 45 min on ice. Cysts of *Heterodera avenae* have been stored successfully at –18°C (Ireholm, 1996).

Examination of Nematode Suspensions

Direct examination

Extracted nematodes can be examined directly under a microscope to the genus level using open counting dishes or fixed capacity, usually 1 ml, covered counting slides (Fig. 4.3). A good stereoscopic microscope with a range of magnifications 10× to 100×, a fairly flat field and good resolution are essential. All or part of the extracted suspension, according to nematode density, is placed in a counting dish/slide and examined under the microscope. When samples are taken with a pipette, it should have a wide outlet to prevent debris or large nematodes

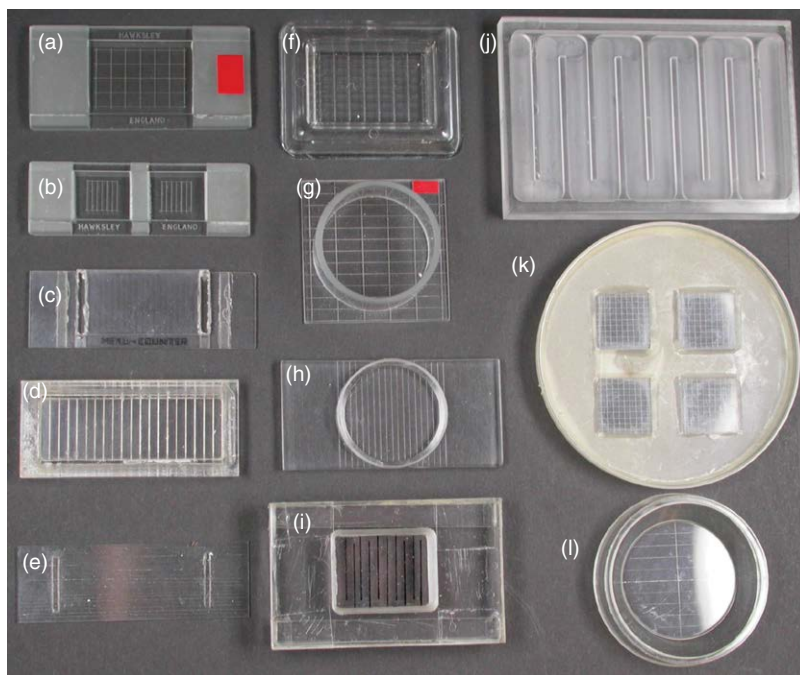


Fig. 4.3. Examples of counting slides/dishes: (a) Peter's 1 ml counting slide in glass, as made by Hawksley, UK; (b) multichamber counting slide in glass, as made by Hawksley; (c) 1 ml counting slide made by MEKU, Germany; (d) 2 ml counting slide in plastic (made at JKI Münster, Germany); (e) microscope slide with ridges to hold a large cover slide, 1 ml volume (made by Sikora, Bonn University, Germany); (f) moulded plastic dish, 5 ml, with sloping sides and ridged grid (made at Rothamsted Experimental Station, UK); (g) glass ring, 38 mm, glued on a glass plate for counting cysts (made at JKI Münster); (h) 2 ml counting slide with sloping sides consisting of a 2 mm high plastic ring glued on a plastic plate of 75 × 37 mm (made by Sikora, Bonn University, Germany); (i) 2 ml counting slide in plastic with a coverglass of 78 × 48 mm, as the bottom to allow examination with an inverse microscope (made at JKI Münster); (j) 10 ml winding-track counting tray in plastic, as made by Nordmeyer and Sikora (at Bonn University, Germany); (k) multichamber counting slide with sloping sides made in paraffin within a 90 mm diameter plastic Petri dish (made at JKI Münster); (l) 50 mm diameter plastic tissue culture Petri dish marked for examination at 20–40×, base lines are cut with a plastic or glass writing knife into the lid. (Photograph courtesy of JKI Münster.)

clogging it. Petri dishes or flat-bottomed Syracuse watch glasses (Shurtleff and Averre, 2000) are often used, and a grid is etched, or scratched with a marking diamond, on the inside of the base to act as a guide when counting. Small disposable tissue culture plastic Petri dishes (5 cm in diameter) that have sloping sides can be used on which a grid is scratched easily with a needle (Fig. 4.3). To be sure of searching over the whole area of the dish, the space between the grid lines should be a little less than the field width of the microscope at the magnification being used. Thus, a dish with an extract containing average size nematodes would be examined at about 50×

and have lines about 3 mm apart. Some workers prefer to examine extracts in a dish with a thin base (e.g. a disposable plastic Petri dish) using the low/medium power objectives of an inverted compound microscope when nematodes can be seen in more detail. Covered counting slide chambers are useful for routine counts when immediate access to nematodes within the suspension is not required. Examples are shown in Fig. 4.3. Counting slides and dishes are in many cases custom made (Doncaster *et al.*, 1967; Southey, 1986); others are commercially available, such as 1 ml covered counting slides from Chalex LLC (www.vetslides.com, accessed

2 November 2017) or 10 ml open counting dishes from Wageningen University (<https://www.wur.nl/en/show/Nematode-counting-dishes-2.htm>, accessed 2 November 2017). A hand tally counter or a multiple bank of counters is an essential aid for counting different genera. For nematode identification to the species level, temporary or permanent slides need to be prepared, which includes handling of the nematodes.

Handling nematodes

There are various methods for handling nematodes. Small batches of nematodes can be selected and transferred from a suspension by using a fine pipette. The modified Hesling's device (Alam, 1990) or the suction device described by Sehgal and Gaur (1988) even allow the selection of individual specimens. However, in most cases, a handling needle is preferred, which is a dissecting needle handle to the end of which is attached with glue a nylon toothbrush bristle, sharpened bamboo splinter, eyebrow hair, fine wire or small wire loop. To 'fish' nematodes, the specimens should be in shallow water, near the centre of the dish, and the lowest convenient microscope magnification used to give the greatest possible depth of focus and working distance. While viewed with the stereoscopic microscope, the handling needle is used to lift the nematode to the surface of the water; the bristle is then held immediately underneath the nematode and flicked up quickly so that the nematode is pulled out through the meniscus. The surface tension can be removed by adding a small drop of detergent. Picking up fixed nematodes from glycerine is generally easier, due to its higher viscosity.

Killing and fixing nematodes

For identification to the species level and permanent storage, nematodes must first be killed, fixed and properly mounted. The following method is recommended for killing and fixing nematodes in one step (Seinhorst, 1966): specimens are concentrated in ~3 ml of water in a 10 ml glass vial, either by centrifuging or by letting them settle and siphoning off the supernatant. The vial is shaken to disperse the nematodes. Fixatives that

can be used are TAF (2 ml of triethanolamine, 7 ml formaldehyde 40%, 91 ml distilled water) or 4:1 FA containing 10 ml formalin (40% formaldehyde), 1 ml glacial acetic acid and distilled water up to 100 ml. If equal amounts of fixative are added to the nematode suspension, the fixative needs to be double strength. This can be made up using half the amount of water indicated above. The fixative is heated to 70–75°C in a small tube held in a water bath of the required temperature for a few minutes, preferably monitored with a thermometer, and added to the nematode suspension. This method gives a very good fixation of glands and gonads. Nuclei tend to expand and are seen more easily. Although specimens appear rather dark as soon as they are fixed, processing to glycerol will eventually clear them. However, fixatives usually cause some shrinkage and/or distortion of the specimen (Grewal *et al.*, 1990). The addition of 2% glycerol to the above means that nematodes can be brought directly from fixative to glycerol by slow evaporation (see below). Also, as noted by Hooper (1987), nematodes stored in vials will eventually end up in glycerol should the fixative evaporate. Nematodes will be spoiled if placed alive into cold fixative. Alcoholic fixatives should be avoided as they usually shrink nematodes. Well-fixed specimens have a smooth outline. Nematodes can be stored in formalin indefinitely. However, due to toxic fumes, all work with formaldehyde must be conducted under the exhaust hood.

Comparing the different methods, Grewal *et al.* (1990) found that killing and fixing with the addition of hot (95°C) TAF produced the least affected specimens compared with FA 4:1. Chakrabarti and Saha (2001) arrived at a similar conclusion using TAF at 50°C. The most lifelike specimens were produced when fixed in TAF and processed to glycerol by the slow method (outlined below) (Grewal *et al.*, 1990; Siddiqi, 2000).

Processing and Mounting Nematodes

In fixed nematodes, much of the internal body contents, especially gonad structure, may be obscured by the granular appearance of the intestine. Specimens can be cleared by processing with lactoglycerol or glycerol, which are also suitable mountants. Lactoglycerol is a solution of equal amounts of lactic acid, glycerol and

distilled water, to which can be added 0.05% acid fuchsin or 0.05% methyl blue to stain the specimen, if required. However, glycerine mounts are preferred. Several techniques exist that allow processing of the specimens through alcohol to glycerine with minimum time and effort (Hooper, 1987). Mounted specimens can deteriorate, and the storage of some representatives in glycerol in vials is recommended.

Glycerol method

Most nematodes are best preserved in anhydrous glycerol. Transfer from the fixative to glycerol can follow a slow or rapid method. The former usually gives better preservation and is therefore recommended if time is not a limiting factor.

Slow method

Remove most of the fixative from preserved specimens in a small dish or deep glass block with a fine pipette, but take care not to draw nematodes inadvertently. Add 3–4 ml of the following solution: anhydrous glycerol, 2 ml; 96% ethanol, 1 ml; distilled water, 90 ml.

Cover the dish loosely and let the sample stand at room temperature for 2–3 weeks or until water and ethanol have all evaporated. The process can be speeded up in an oven at 30–40°C, but the container needs to be well covered to ensure that the evaporation takes several days. If evaporation is too rapid, the nematodes shrink and become distorted. Golden (in Hooper, 1970) recommends the addition of a few drops of picric acid, which helps to prevent clearing and fading of nematode stylets and the growth of moulds. If completed, the nematodes are in pure glycerol and can be stored indefinitely or used for preparing permanent microscope slides. Note that nematodes processed to glycerol are very soft and should be handled carefully, preferably using a mounted eyebrow hair or similar soft bristle.

Rapid method (Seinhorst, 1962)

Fixed specimens are transferred to a small, concave glass dish of 2–4 ml capacity containing about 0.5 ml of the following solution: 96% ethanol, 20 ml; glycerol, 1 ml; distilled water, 79 ml.

The dish with nematodes is placed into a closed glass vessel containing an excess (e.g. 1/10 volume of the vessel) of 96% ethanol. The dish is supported above the ethanol on a platform or grid. After a minimum of 12 h in an oven at 40°C, the specimens will be in a mixture of mainly ethanol, with some glycerol. Remove the dish from the vessel; excess ethanol can be withdrawn using a pipette, and add a solution of five parts glycerol and 95 parts of 96% ethanol. Then place the dish in a partly closed Petri dish in an oven at 40°C until the ethanol has evaporated. This should take at least 3 h; the nematodes are then in pure glycerol and should be mounted immediately in anhydrous glycerol.

Mounting nematodes

The nematodes are best mounted on thin microscope glass slides (25 × 76 mm) using 19 mm diameter round cover slips. Supports (e.g. stainless-steel wire, tungsten filaments of calibrated diameter, glass fibre or beads) with similar thickness as the nematodes are required to prevent deformation of the specimens from the weight of the cover glass.

For permanent mounts, a very small drop of anhydrous glycerol (heated for 4 h at 40°C in an oven) is placed in the centre of a clean microscope slide and nematodes of about equal diameter are transferred to it, using a handling needle, and arranged in the centre of the drop so that they are touching the slide surface, not floating. Three cover glass supports should be arranged around the nematodes. Paraffin wax of melting point 60–65°C is used as seal, but also provides additional support. A wax ring is prepared using a copper tube (15 mm in diameter, heatproof handle) heated in a flame, dipped in paraffin wax and applied to the centre of the slide surrounding the mountant. A clean cover glass (19 mm diameter circle No 1) held with fine forceps is lowered on to the drop. A mounted needle held in the other hand can be used to help prevent the cover glass from sliding sideways when it is applied. It helps to prevent air bubbles from being trapped if the drop is kept as hemispherical as possible before applying the cover glass. The slide is placed on a hotplate at 65°C for a few seconds. As soon as the wax melts, press lightly with a mounted needle on the cover glass

to make sure it has settled far enough; thick mounts prevent oil immersion objectives being used. The wax will set rapidly when the slide is placed on a cool surface. A secondary seal is desirable to prevent drying out and to prevent immersion oil dissolving the wax, such as Permunt (Thermo Fisher Scientific, USA), Corseal (Sabir, 1997) or Glyceel (Bates, 1997), which are excellent, or nail varnish. Seal the cover glass using a small soft brush, with a thick but fairly narrow band of the sealant, making sure there is sufficient on the cover glass as well as on the slide. Repeat the process when the first ring has dried, to give a good seal.

Instead of a wax ring, Siddiqi (2000) recommends the use of three small lumps of wax, each about the size of the mounting drop, arranged around the drop, and the cover glass is placed on the lumps and the slide then heated. The wax melts, allowing the cover glass to settle down, and confines the glycerol to the centre of the mount. It is important to retain a hemispherical drop of mountant before applying the cover glass, or the wax may swamp the specimens. Supports, however, remain useful to prevent deformation of the nematodes.

Posterior cuticular patterns of *Meloidogyne* spp.

The cuticular markings surrounding the vulva and anus (posterior cuticular pattern, or 'perineal' pattern) of females of *Meloidogyne* spp. are used in their identification (Taylor *et al.*, 1955; Franklin, 1962). Fresh or fixed galled roots are stained in cotton-blue lactophenol or lactoglycerol. Females stained in fresh root material are preferable, because their body contents are removed more easily (Franklin, 1962). About 20 females are dissected out and transferred, using fine-pointed forceps, to 45% lactic acid on a transparent perspex slide or plastic Petri dish cover. Working at a magnification of at least 32 $\times$, preferably more, the swollen female is speared at the neck end with a very sharp, fine needle and held so that the posterior end can be cut off with an oculist's scalpel or sharp Borradaile needle. A hypodermic needle mounted on a handle also serves as a useful cutting tool. The inner tissue is removed carefully by brushing lightly with a flexible bristle. The cuticle is trans-

ferred to a drop of glycerol, where it is trimmed to a size slightly greater than the pattern, which is then transferred to a drop of glycerol on a clean glass slide. The posterior patterns, outside uppermost, are arranged in one or two neat rows, and a cover glass is applied and sealed. Supports are optional. At least ten specimens from a population should be examined. The patterns can usually be seen satisfactorily at a magnification of about 500 $\times$, but for species having small or indistinct patterns, an oil immersion objective and higher magnification may be needed.

As noted by Taylor (1987), the lip region shape and the position of the excretory pore in mature females are an aid to the identification of *Meloidogyne* spp. Gerber and Taylor (1988) give details of preparation and mounting so as to show the anterior end and perineal pattern on one specimen. The preparation is similar to that described above for perineal patterns only, but the mature female is pierced once or twice in the mid-body region and the body contents squeezed out carefully. The female is then orientated with the perineal pattern to one side and, using a fine scalpel or hypodermic needle, the posterior quarter of the body, without the pattern, is cut away, taking care not to damage the pattern. The prepared specimens are then mounted in glycerol, with the cut opening underneath and the perineal pattern uppermost. For additional information on preparation methods for culturing and identification of *Meloidogyne* spp., see Barker *et al.* (1985) or Jepson (1987).

Vulval cones of cyst nematodes

The structure of the vulva, fenestra and associated internal structures as well as the general shape of cysts are used for identifying cyst nematodes (e.g. *Globodera* and *Heterodera*) (Hesling, 1978). A detailed protocol for the preparation of vulval cones of cyst nematodes is given by Subbotin *et al.* (2010). Dry cysts should be soaked in water for up to 24 h before dissection. Place a moist cyst on a perspex slide on the stage of a stereomicroscope and cut the posterior end off so that the fenestral area is in the centre of the cut piece. Trim the cut end so that it is no more than 5–10 times the fenestral area. Using fine forceps and a flexible probe (e.g. eyebrow or fine

toothbrush bristle), clean away any adhering body contents, e.g. eggs, taking particular care not to damage the structures associated with the vulva. Thick-walled and heavily pigmented species, bleached for a few minutes in H_2O_2 , often have more visible structures. Avoid overbleaching. Wash the cleaned vulval cones in distilled water and then pass through 70, 95 and 100% ethanol to clove oil. After clearing in clove oil, mount in Canada balsam. Support the cover glass with pieces of glass rod or broken cover glass to prevent distortion of the specimen. Vulval cones may also be mounted in 'Euparal', after passage through 70% ethanol and isobutanol, or directly in glycerine and sealed.

A simpler method for the examination of the vulval cone of mature *Heterodera* cysts is described by Esser (1988). Place a block of 1.7% water agar (15 mm × 15 mm × 2 mm high) on a slide and make a small 1 mm deep cavity slightly less than the diameter of the cyst with a fine needle. Gently press the cyst into the cavity with the anterior end down until the vulva region of the cyst is at the same level as the agar surface. Add a small drop of water to a 15 mm cover slip, which is inverted and dropped over the embedded cyst, which can then be viewed under the microscope. Correia and Abrantes (1997) describe an improved technique for mounting *Heterodera* cysts in glycerine agar.

Computerized systems

Image analysis systems can assist with the examination of nematode samples by counting nematodes in a suspension (Been *et al.*, 1996) or with automatic recognition of nematodes (Fernandez-Valdivia *et al.*, 1989). Furthermore, computerized keys can help with the identification of species (Viscardi and Brzeski, 1993, 1995). An example of a computerized key is freely accessible on the website of the University of Nebraska, Lincoln (<http://nematode.unl.edu/key/nemakey.htm>, accessed 2 November 2017).

Molecular Diagnostics

Most methods of nematode diagnostics have their limitations. Species identification based on

morphological and morphometrical characters requires much skill, but can often be inconclusive for individual nematodes. Isozyme or total protein analyses are relatively fast ways to identify root knot or cyst-forming nematode species. Although differences in isozyme or protein patterns show significant consistency and are useful for species identification, reliable results can only be obtained with nematodes of specific developmental stage. DNA-based diagnostics do not rely on the express products of the genome, and are independent of environmental influence or developmental stage. Recent progress in nematode diagnostics has been achieved due to introducing the polymerase chain reaction (PCR), a powerful method with widespread application in many biological fields (Fig. 4.4). A single nematode, egg, or even a part of the nematode body, can be identified using this technology. The majority of PCR-based techniques developed for nematode diagnostics indicate differences of the ribosomal RNA (rRNA) or mitochondrial DNA (mtDNA) gene sequences.

rRNA and mtDNA genes

The rRNA genes are arranged as tandem repeats, with several hundred copies per genome. Each repeat includes the small subunit (SSU) gene, or 18S gene, the 5.8S gene and the large subunit (LSU) gene, or 28S gene, the spacer region between the subunit and 5.8S gene, called the internal transcribed spacers (ITS1 and ITS2), and between the gene cluster, called the intergenic spacer (IGS). In the root knot nematodes, the 5S gene is found in the IGS. The 18S gene evolves relatively slowly and is useful for comparison of distantly related groups, whereas ITS and IGS are considerably more variable and can be used to distinguish species or subspecies. Some regions of the 28S gene are also useful for species differentiation.

MtDNA is a circular, double-stranded, closed, small structure that is present in large copy numbers in the cell. Rapid evolution rates of specific genes in the mtDNA, which evolve ten times faster and more than nuclear genes, result in accumulated sequence polymorphism. This allows this molecule to be used as a useful marker for differentiation of nematode populations and of closely related species. For example, sequences

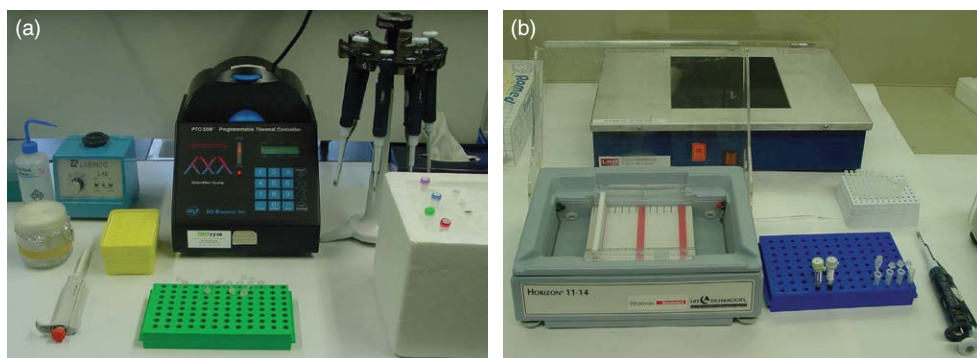


Fig. 4.4. Equipment required for PCR (a), electrophoresis and visualization of the PCR product on agarose gel (b).

of intergenic spacer, large subunit of the rRNA, mitochondrial cytochrome c oxidase 1 (COI) and NADH dehydrogenase subunit 5 (NAD5) genes can be used successfully for differentiation of root knot nematodes from the *M. incognita* group (Powers and Harris, 1993; Pagan *et al.*, 2015; Janssen *et al.*, 2016).

DNA extraction

The first step in molecular diagnostic procedures is the preparation of the template DNA (see Examples 1 and 2 below). Several protocols for the extraction of nucleic acids from nematodes are available (Curran *et al.*, 1985; Caswell-Chen *et al.*, 1992; Blok *et al.*, 1997). Some of these allow the isolation of microgram quantities of pure genomic DNA. However, because only small quantities of starting DNA are required for PCR amplification, simplified and rapid procedures can generally be used (Harris *et al.*, 1990; Subbotin *et al.*, 2000; Waeyenberge *et al.*, 2000; Floyd *et al.*, 2002). Using different extraction methods and commercial kits, nematode DNA can be obtained directly from soil samples (Nazar *et al.*, 1995; Waite *et al.*, 2003). Furthermore, extraction of DNA from formalin-fixed materials or nematodes embedded in glycerine on slides provides a new opportunity for molecular examination of reference materials (Thomas *et al.*, 1997; Rubtsova *et al.*, 2005).

Example 1: protocol for DNA extraction using proteinase K with worm lysis buffer (WLB) (Waeyenberge *et al.*, 2000).

1. Select a single or several nematodes and place in a 10 μ l drop of double-distilled water on a glass slide under the dissecting microscope.
2. Cut nematodes into three or four pieces with a needle or scalpel.
3. Transfer worm bits with water to a sterile 0.2 ml Eppendorf tube containing 8 μ l of WLB (500 mM KCl, 100 mM Tris-HCl pH 8.3, 15 mM $MgCl_2$, 10 mM dithiothreitol (DTT); 4.5% Tween-20) and 2 μ l of proteinase K (600 μ g/ml).
3. Freeze at -80°C for 10 min.
5. Incubate at 65°C for 1 h and then heat at 95°C for 15 min.
6. Centrifuge for 1 min at maximum speed to remove debris. Use 1–4 μ l of the supernatant in the PCR.

Example 2: protocol for DNA extraction using NaOH (Floyd *et al.*, 2002).

1. Transfer individual nematodes directly into 20 μ l of 0.25 M NaOH in a 0.2 ml Eppendorf tube and keep at room temperature from several minutes to several hours.
2. Heat the lysate for 3 min at 95°C .
3. Add 4 μ l of HCl and 10 μ l of 0.5 M Tris-HCl buffered at pH 8.0 to neutralize the base.
4. Add 5 μ l of 2% Triton X-100.
5. Heat the lysate for 3 min at 95°C .
6. Use 0.5–2.0 μ l of lysate for the PCR.

PCR

This enzymatic reaction allows *in vitro* amplification of target DNA fragments by up to a billion-fold from complex DNA samples within a

test tube. Any nucleic acid sequence can be detected by PCR amplification. The method requires a DNA template containing the region to be amplified, two oligonucleotide primers flanking this target region (Table 4.1), DNA polymerase and deoxyribonucleotide triphosphates (dNTPs) mixed in buffer containing magnesium ions (MgCl_2) (Example 3). The PCR is performed in tubes, with final volumes of 20–100 μl . The PCR procedure consists of a succession of three steps, which are determined by temperature condition: template denaturation at 95°C for 3–4 min, primer annealing at 55–60°C for 1–2 min and extension at 72°C for 1–2 min. The PCR is carried out for 30–40 cycles in a thermocycler with programmed heating and cooling. Finally, PCR products are separated electrophoretically, according to their size, on agarose gels and visualized by ethidium bromide under ultraviolet (UV) light. Once identified, nematode target DNA generated by PCR amplification can be characterized further by various analyses: restriction fragment length polymorphism (RFLP), single-strand conformation polymorphism or sequencing.

Example 3: PCR protocol.

1. Add a DNA suspension to the Eppendorf tube containing a PCR mixture with 5 μl of 10× PCR buffer, 10 μl of Q-solution, 1 μmol of dNTP mixture (10 mM each) (*Taq* PCR Core Kit, Qiagen), 0.5 μl of each primer, 1 U of *Taq* polymerase, and double-distilled water to a final volume of 50 μl .
2. Place the tube in the PCR machine with an initial denaturation at 94°C for 4 min, 35 cycles of 94°C for 1 min, 55°C for 1.5 min, 72°C for 2 min and a final elongation step at 72°C for 10 min.
3. Run 2–5 μl of PCR product on a 0.8–1% agarose gel for 30–60 min at 90–100 V.

PCR-RFLP

Variation in sequences in PCR products can be revealed by restriction endonuclease digestion. The PCR product obtained from different species or populations can be digested by a restriction enzyme and the resulting fragment is separated by electrophoresis (Example 4). If there is some difference in sequences situated within the restriction site of the enzyme, the digestion of the PCR products will lead to different electrophoretic profiles. It has been shown that the comparison of restriction patterns derived from amplified ITS regions

is a very useful approach to distinguish species and populations. PCR-RFLP protocols are available for all relevant genera, often even with several protocols for one genus. Just a few examples are provided here, such as for *Aphelenchoides* (Ibrahim *et al.*, 1994), cyst-forming nematodes (Thiéry and Mugniéry, 1996; Szalanski *et al.*, 1997; Subbotin *et al.*, 2000) (Fig. 4.5), *Ditylenchus* (Ibrahim *et al.*, 1994), *Hemicycliophora* (Subbotin *et al.*, 2014), *Longidorus* (Subbotin *et al.*, 2013), *Nacobbus* (Reid *et al.*, 2003), *Pratylenchus* (Waeyenberge *et al.*, 2000), *Radopholus* (Fallas *et al.*, 1996), root knot nematodes (Zijlstra *et al.*, 1995; Schmitz *et al.*, 1998), *Trichodorus* (Kumari and Subbotin, 2012), *Tylenchulus* (Tanha Maafi *et al.*, 2012) and *Xiphinema* (Vrain *et al.*, 1992). Comparison of RFLP profiles from newly obtained samples with those from known species provide a quick tool for nematode identification. PCR-RFLPs are especially suited to identify nematodes of monospecific probes; this strategy does not allow mixed species populations to be identified.

Example 4: RFLP protocol.

1. Add 2–8 μl of PCR product to an Eppendorf tube containing 1.0 μl of 10× restriction enzyme buffer, 1 μl of restriction enzyme and double-distilled water to a final volume of 10 μl .
2. Place the tube in a water bath at 37°C (or other temperature required for digestion) for 1–12 h.
3. Centrifuge the tube for 30 s at maximum speed.
4. Run the reaction mixture on a 1.5% agarose gel in 1× TBE for 60–90 min at 90–100 V.

The restriction enzymes recommended for species identification are *AluI*, *AvaI*, *Bsh1236I*, *BsuRI*, *CfoI*, *HinII*, *MvaI*, *RsaI* and *PstI* for cyst-forming nematodes (Fig. 4.5), and *AluI*, *DraI*, *HinII*, *MspI*, *PvuII* and *RsaI* for root knot nematodes.

Sequencing

Direct sequencing of PCR products or sequencing of cloned PCR fragments provides full characterization of amplified target DNA. One of the first applications of PCR in plant nematology was presented by Ferris *et al.* (1993), who used the ITS rDNA sequences to establish the taxonomic and phylogenetic relationships of cyst-forming nematodes. The sequences of the ITS regions, fragments of 18S and 28S of rRNA genes, have been examined for a wide range of plant parasitic nematodes.

Table 4.1. Universal primers frequently used for nematode diagnostics.

Code	Primer (5'–3')								Amplified region	Reference
C2F3	GGT	CAA	TGT	TCA	GAA	ATT	TGT	GG	3' of COII to 16S mitochondrial genes	Powers and Harris (1993)
1108	TAC	CTT	TGA	CCA	ATC	ACG	CT			
18S	TTG	ATT	ACG	TCC	CTG	CCC	TTT			
rDNA1.58S	GCC	ACC	TAG	TGA	GCC	GCG	CA			
18S	TTG	ATT	ACG	TCC	CTG	CCC	TTT		ITS1–5.8S–ITS2 region of rDNA	Vrain <i>et al.</i> (1992)
26S	TTT	CAC	TCG	CCG	TTA	CTA	AGG			
F194	CGT	AAC	AAG	GTA	GCT	GTA	G			
F195	TCC	TCC	GCT	AAA	TGA	TAT	G			
SSU18A	AAA	GAT	TAA	GCC	ATG	CAT	G		18S gene of rDNA	Blaxter <i>et al.</i> (1998)
SSU26R	CAT	TCT	TGG	CAA	ATG	CTT	TCG			
D2A	ACA	AGT	ACC	GTG	AGG	GAA	AGT			
D3B	TCG	GAA	GGA	ACC	AGC	TAC	TA			
TW81	GTT	TCC	GTA	GGT	GAA	CCT	GC	TG	D2–D3 expansion segments of 28S gene of rDNA	De Ley <i>et al.</i> (1999)
AB28	ATA	TGC	TTA	AGT	TCA	GCG	GGT			
									ITS1–5.8S–ITS2 region of rDNA	Joyce <i>et al.</i> (1994)

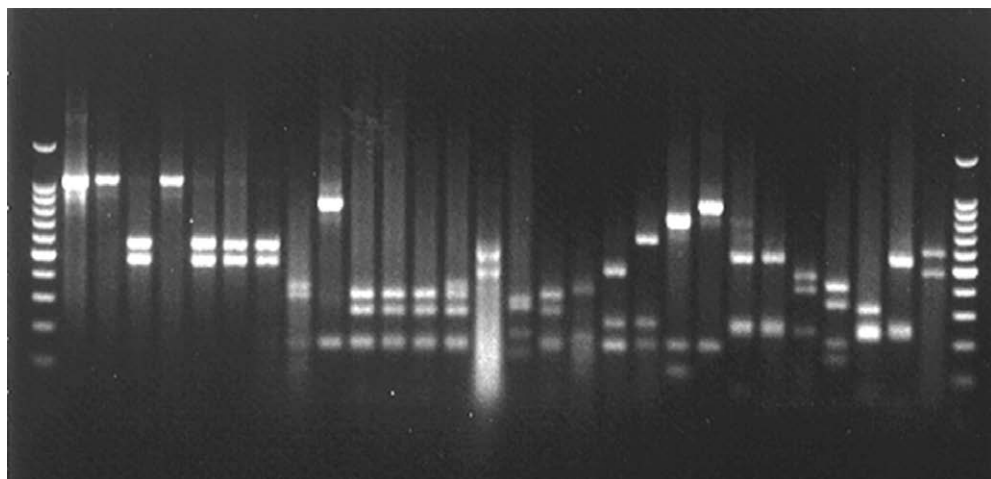


Fig. 4.5. RFLP patterns obtained after *AluI* digestion of the amplified PCR product of the ITS-rDNA for cyst-forming nematodes. L, 100 bp DNA ladder; U, unrestricted PCR product; 1, 2, *Heterodera avenae*; 3, *Heterodera arenaria*; 4, *Heterodera filipjevi*; 5, *Heterodera aucklandica*; 6, *Heterodera ustinoi*; 7, *Heterodera latipons*; 8, *Heterodera hordecalis*; 9, *Heterodera schachtii*; 10, *Heterodera trifolii*; 11, *Heterodera medicaginis*; 12, *Heterodera ciceri*; 13, *Heterodera salixophila*; 14, *Heterodera oryzicola*; 15, *Heterodera glycines*; 16, *Heterodera cajani*; 17, *Heterodera humuli*; 18, *Heterodera ripae*; 19, *Heterodera fici*; 20, *Heterodera litoralis*; 21, *Heterodera carotae*; 22, *Heterodera cruciferae*; 23, *Heterodera* sp.; 24, *Heterodera cyperi*; 25, *Heterodera goettingiana*; 26, *Heterodera urticae*; 27, *Meloidodera alni*. (From Subbotin et al., 2000.)

The comparison of newly obtained sequences from samples with those published or deposited in the GenBank (www.ncbi.nlm.nih.gov/genbank/) is a most reliable approach for molecular identification. Increasing numbers of deposited nematode rDNA sequences, as well as decreasing costs for sequence analyses, will allow wider application of this still rather expensive procedure for routine nematode diagnostics in the future.

PCR with species-specific primers

PCR with specific primer combinations or multiplex PCR constitute a major development in DNA diagnostics and allow the detection of one or several species in a nematode mixture by a single PCR test, thus decreasing diagnostic time and costs. Species-specific primers are designed based on the broad knowledge of sequence divergence of the target DNA region in many populations of the same species and in closely related species. This knowledge allows the detection of populations with small differences in sequences, and avoids the amplification of an identical specific fragment

in other species. The principle of this method is the alignment of the sequences from target and non-target organisms and the selection of primer mismatches to non-target organisms, but it shows sufficient homology for efficient priming and amplification of the target organism. This diagnostic tool has been developed for the identification of many agriculturally important plant nematodes (Fig. 4.6; Table 4.2). The multiplex PCR with specific primers for the identification of several nematode targets in one assay is limited by the number of primer pairs that can be used in a single reaction and the number of bands that can be identified clearly without giving false-positive results. This technique requires precise optimization of the reaction conditions for the primer sets used simultaneously in the test.

Reverse dot-blot hybridization

This technique involves the use of PCR for simultaneous amplification and labelling of target DNA to generate digoxigenin-dUTP-labelled amplicons, which are hybridized to specific immobilized

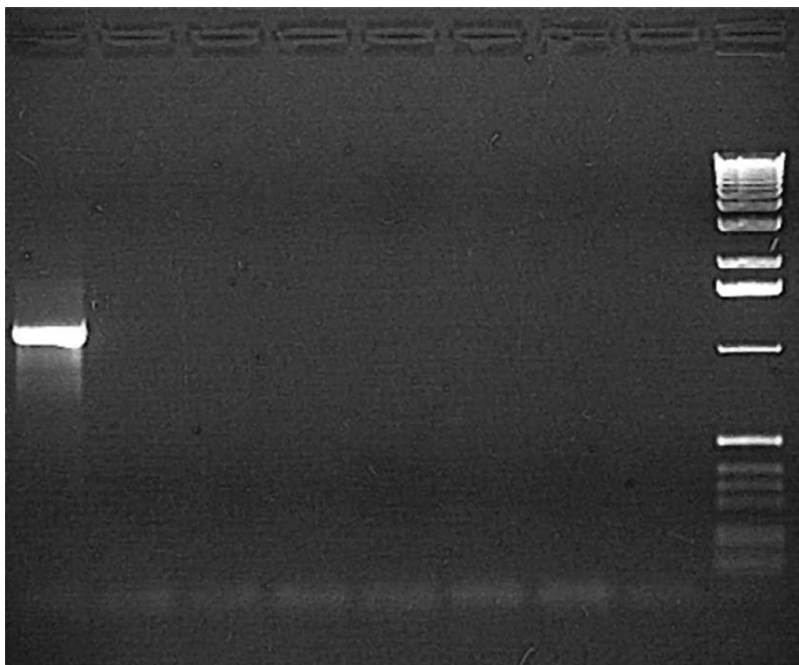


Fig. 4.6 Amplification product of PCR with species-specific primer Finc/Rinc for *Meloidogyne incognita*. I, *Meloidogyne incognita*; J, *Meloidogyne javanica*; A, *Meloidogyne arenaria*; M, *Meloidogyne mayaguensis*; H, *Meloidogyne hapla*; C, *Meloidogyne chitwoodi*; F, *Meloidogyne fallax*; W, no template DNA control; S, size marker. (From Zijlstra *et al.*, 2000.)

oligonucleotide probes on a membrane. This approach can be used for the simultaneous identification of many different nematodes from a single sample. Uehara *et al.* (1999) demonstrated that this technology could be used for the identification of *Pratylenchus* species (Fig. 4.7).

RAPD-PCR

In contrast to the above-mentioned classical PCR method, the random amplified polymorphic DNA PCR (RAPD-PCR) or PCR with arbitrary primer (AP-PCR) does not require any information on the primer design. This PCR technology uses a single random primer of about ten nucleotides long, approximately 50% GC rich and lacking any internal inverted repeats. By lowering the annealing temperature during the amplification cycle, the primer anneals at random in the genome, allowing the synthesis of highly polymorphic amplification products. RAPD-PCR distinguishes nematode species and subspecies

for root knot nematodes (Cenis, 1993; Blok *et al.*, 1997; Cofcewicz *et al.*, 2005) and cyst-forming nematodes (Caswell-Chen *et al.*, 1992; Thiéry *et al.*, 1997) (Fig. 4.8). However, the reproducibility of the results is the most critical point for application of this technique for diagnostic purposes. Specific sequences for certain species or races, called SCARs (sequence characterized amplified regions), can be derived from RAPD fragments and further used to design species-specific primers.

AFLP

The amplified fragment length polymorphism (AFLP) technique was developed by Vos *et al.* (1995) and was based on the selective amplification of genomic restriction fragments. AFLP involves three steps: (i) digestion of DNA with two restriction enzymes and ligation of specific adapters to the restriction fragments; (ii) PCR amplification of a subset of the restriction/adaptor

Table 4.2. Species-specific primers developed for identification of some nematodes.

Species	Primer set (5'–3')	Amplicon length	Reference
<i>Ditylenchus destructor</i>	D2 TGG ATC ACT CGG CGG CTC GTA GA D1 ACT GCT CTG CGT TTG GCT TCA	346 bp	Liu <i>et al.</i> (2007)
<i>Heterodera latipons</i>	Hlat-actF ATG CCA TCA TTA TTC CTT Hlat-actR ACA GAG AGT CAA ATT GTG	204 bp	Toumi <i>et al.</i> (2013)
<i>Heterodera filipjevi</i>	HfITS-F1 CCC GTC TGC TGT TGA GA HfITS-R1 ACC TCA GGC TTT TAT TAT CAC	170 bp	Yan and Smiley (2010)
<i>Heterodera avenae</i>	HalTS-F6 ATG CCC CCG TCT GCT GA HalTS-R4 GAG CGT GCT CGT CCA AC	242 bp	Yan and Smiley (2010)
<i>Globodera pallida</i>	PITSp4 ACA ACA GCA ATC GTC GAG ITS5 GGA AGT AAA AGT CGT AAC AAG G	265 bp	Bulman and Marshall (1997)
<i>Meloidogyne arenaria</i>	Far TCG GCG ATA GAG GTA AAT GAC Rar TCG GCG ATA GAC ACT ACA ACT	420 bp	Zijlstra <i>et al.</i> (2000)
<i>Meloidogyne chitwoodi</i>	Fc TGG AGA GCA GCA GGA GAA AGA Rc GGT CTG AGT GAG GAC AAG AGT A	800 bp	Zijlstra (2000)
<i>Meloidogyne exigua</i>	Ex-D15-F CAT CCG TGC TGT AGC TGC GAG Ex-D15-R CTC CGT GGG AAG AAA GAC TG	562 bp	Randing <i>et al.</i> (2002)
<i>Meloidogyne hapla</i>	Fh TGA CGG CGG TGA GTG CGA Rh TGA CGG CGG TAC CTC ATA G	610 bp	Zijlstra (2000)
<i>Meloidogyne incognita</i>	Finc CTC TGC CCA ATG AGC TGT CC Rinc CTC TGC CCT CAC ATT AGG	1200 bp	Zijlstra <i>et al.</i> (2000)
<i>Meloidogyne paranaensis</i>	Par-C09-F GCC CGA CTC CAT TTG ACG GA Par-C09-R CCG TCC AGA TCC ATC GAA GTC	208 bp	Randing <i>et al.</i> (2002)
<i>Meloidogyne arabicida</i>	ar-A12F TCG GCG ATA GTA CGT ATT TAG CG ar-A12R TAG TGA TTT CGG CGA TAG GC	300 bp	Correa <i>et al.</i> (2013)
<i>Meloidogyne ethiopica</i>	meth-F ATG CAG CCG CAG GGA ACG TAG TTG meth-R TGT TGT TTC ATG TGC TTC GGC ATC	350 bp	Correa <i>et al.</i> (2014)
<i>Meloidogyne enterolobii</i>	MK7-F GAT CAG AGG CGG GCG CAT TGC GA MK7-R CGA ACT CGC TCG AAC TCG AC	520 bp	Tigano <i>et al.</i> (2010)

Continued

Table 4.2. Continued.

Species	Primer set (5'–3')	Amplicon length	Reference
<i>Meloidogyne naasi</i>	N-ITS CTC TTT ATG GAG AAT AAT CGT R195 CCT CCG CTT ACT GAT ATG	433 bp	Zijlstra <i>et al.</i> (2004)
<i>Pratylenchus penetrans</i>	PNEG ATG AAA GTG AAC ATG TCC TC D3B TCG GAA GGA ACC AGC TAC TA	278 bp	Al-Banna <i>et al.</i> (2004)
<i>Pratylenchus vulnus</i>	PVUL GAA AGT GAA CGC ATC CGC AA D3B TCG GAA GGA ACC AGC TAC TA	287 bp	Al-Banna <i>et al.</i> (2004)
<i>Rotylenchus robustus</i>	TW81 GTT TCC GTA GGT GAA CCT GC R_rob GAC GTG GAC ATC ATA CAG TC	438 bp	Cantalapiedra-Navarrete <i>et al.</i> (2013)
<i>Tylenchulus semipenetrans</i>	TW81 GTT TCC GTA GGT GAA CCT GC Sem_spec GGA CTC TGC TCA ACC TGG TAG A	113 bp	Tanha Maafi <i>et al.</i> (2012)
<i>Xiphinema index</i>	I27 GAG TCG TAA CGT TTC TCG TCT ATC AGG A-ITS1 GAA TAG CCA CCT AGT GAG CCG AGCA	340 bp	Wang <i>et al.</i> (2003)

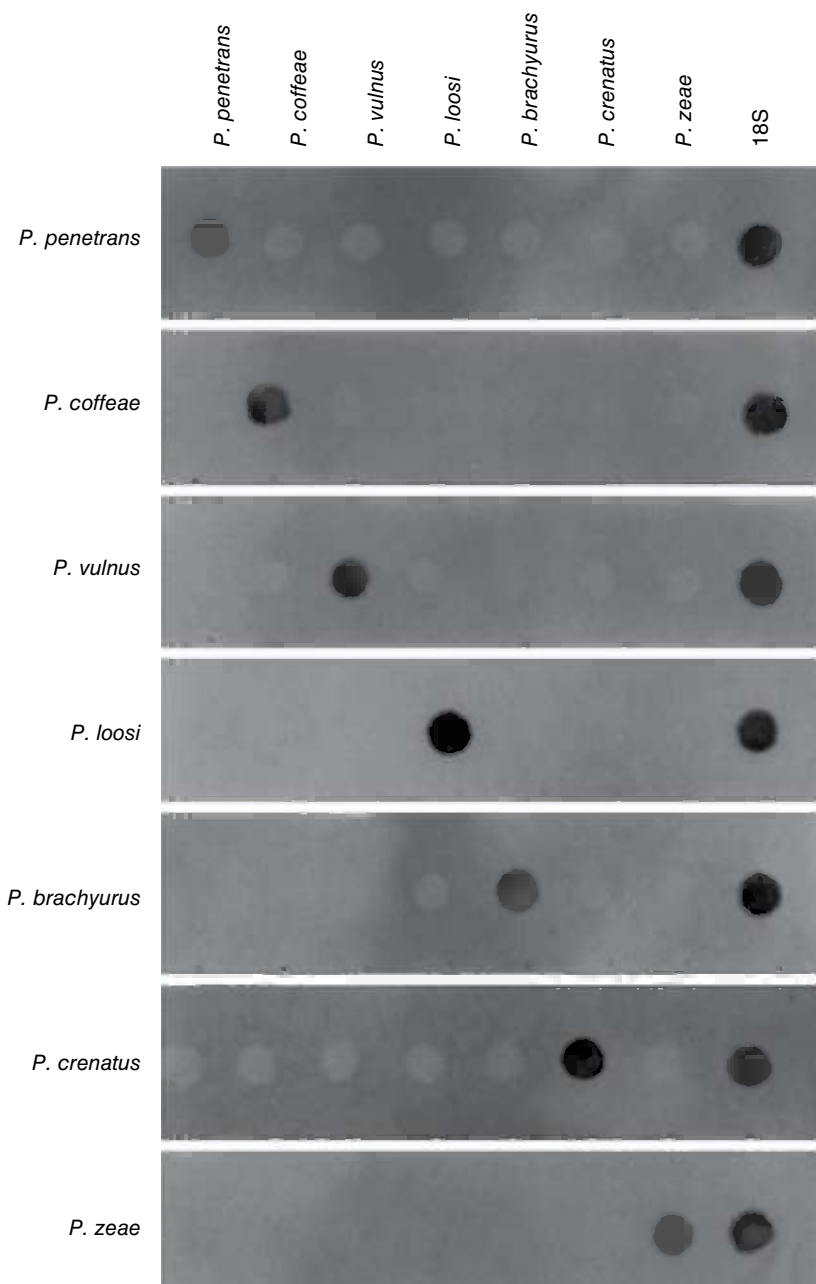


Fig. 4.7. Reverse dot-blot hybridization with immobilized specific oligonucleotides. The *Pratylenchus* species listed on the left were used for each hybridization. (From Uehara *et al.*, 1999.)

fragments under stringent conditions; (iii) gel electrophoresis analysis of the amplified restriction fragments. The AFLP technique has several advantages over RAPD in that it produces results

that are highly reproducible and has higher resolutions generating many more amplified fragments. AFLP fingerprinting has been applied successfully for the evaluation of inter- and

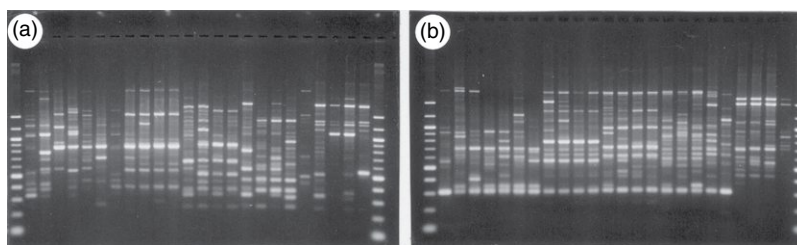


Fig. 4.8. RAPD patterns of 26 populations of the *Heterodera avenae* complex. Primers: (a) A-16; (b) A-18. Populations: 1, *H. avenae* (Taaken, Germany); 2, *H. avenae* (Santa Olalla, Spain); 3, *H. avenae* (Çukurova Ebene, Turkey); 4, *H. avenae* (Saudi Arabia); 5, *H. avenae* (Ha-hoola, Israel); 6, *H. avenae* (Israel); 7, *H. avenae* (near Delhi, India); 8, *Heterodera australis* (South Australia, sample 3); 9, *H. australis* (Beulah, Australia); 10, *H. australis* (Victoria, Australia); 11, *H. australis* (Yorke Peninsular, Australia); 12, *Heterodera mani* (Bayern, Germany); 13, *H. mani* (Heinsberg, Germany); 14, *H. mani* (Andernach, Germany); 15, *H. mani* (Germany); 16, *Heterodera pratensis* (Missunde, Germany); 17, *H. pratensis* (Östergaard, Germany); 18, *H. pratensis* (Lindhöft, Germany); 19, *H. pratensis* (Lenggries, Germany); 20, *Heterodera aucklandica* (One Tree Hill, New Zealand); 21, *Heterodera filipjevi* (Saratov, Russia); 22, *H. filipjevi* (Akenham, England); 23, *H. filipjevi* (Torralba de Calatrava, Spain); 24, *H. filipjevi* (Selçuklu, Turkey). M, 100 bp DNA ladder (Biolab). (From Subbotin *et al.*, 2003.)

intraspecific genetic variation of cyst-forming nematodes (Folkertsma *et al.*, 1996; Marché *et al.*, 2001), root knot nematodes (Semblat *et al.*, 1998) and stem nematodes (Esquibet *et al.*, 2003).

DNA bar coding

The bar-coding technique is based on the idea that a particular nucleotide sequence from a common gene can serve as a unique identifier for every species, and a single piece of DNA can identify all life forms on earth. DNA bar coding first came to the attention of the scientific community when 'Biological identifications through DNA barcodes' was published, in which the authors proposed a new system of species identification and discovery using a 648-bp region of the mitochondrial cytochrome c oxidase subunit I (COI) gene as a standard bar code in the animal kingdom (Hebert *et al.*, 2003). There are considerable debates among taxonomists about DNA bar-code application. Floyd *et al.* (2002) were the first to develop a 'molecular operation taxonomic unit' approach when they applied a molecular bar code, derived from single-specimen PCR and sequencing of the 5' segment of the 18S rRNA gene, to estimate nematode diversity in Scottish grassland. Further studies showed that in some cases the 18S rRNA gene did not contain

sufficient resolution for nematode identification to species level. Moreover, a single bar-code region may be insufficient for the identification of the majority of nematodes, and presently several markers (18S rRNA, D2–D3 of 28S rRNA, ITS rRNA, COI and other genes) are proposed and used for nematode bar coding. The markers should fit three criteria: (i) show significant species-level genetic variability and divergence; (ii) be an appropriately short sequence length so as to facilitate DNA extraction and amplification; (iii) contain conserved flanking sites for developing universal primers. It is important to note that DNA bar coding is only as good as the reference database, and it can only be used to identify species already catalogued. DNA bar coding will be also most reliable for the identification of putative new species, but only for species groups whose genetic diversity has been well surveyed.

Presently, the results of many nematode DNA bar-coding projects are compiled in a central integrative bioinformatics platform – BOLD (Barcode of Life Data Systems, 2009) – that supports all phases of the analytical pathway, from specimen collection to tightly validated bar-code library, and can also accommodate externally produced sequences, either through direct submission or regular incorporation of GenBank sequences (Ratnasingham and Hebert, 2007, 2013).

Real-time PCR

A real-time polymerase chain reaction is a laboratory technique that monitors the amplification of a targeted DNA molecule using sequence-specific primers, fluorescent probes or fluorescent DNA-binding dyes. Real-time PCR is able to

quantify the amount of DNA in a sample. This technique indirectly measures the nematode number by assuming that the number of target DNA copies in the sample is proportional to the number of targeted nematodes (Fig. 4.9). Many real-time fluorescent PCR chemistries exist, but the most widely used are SYBR Green I dye-based

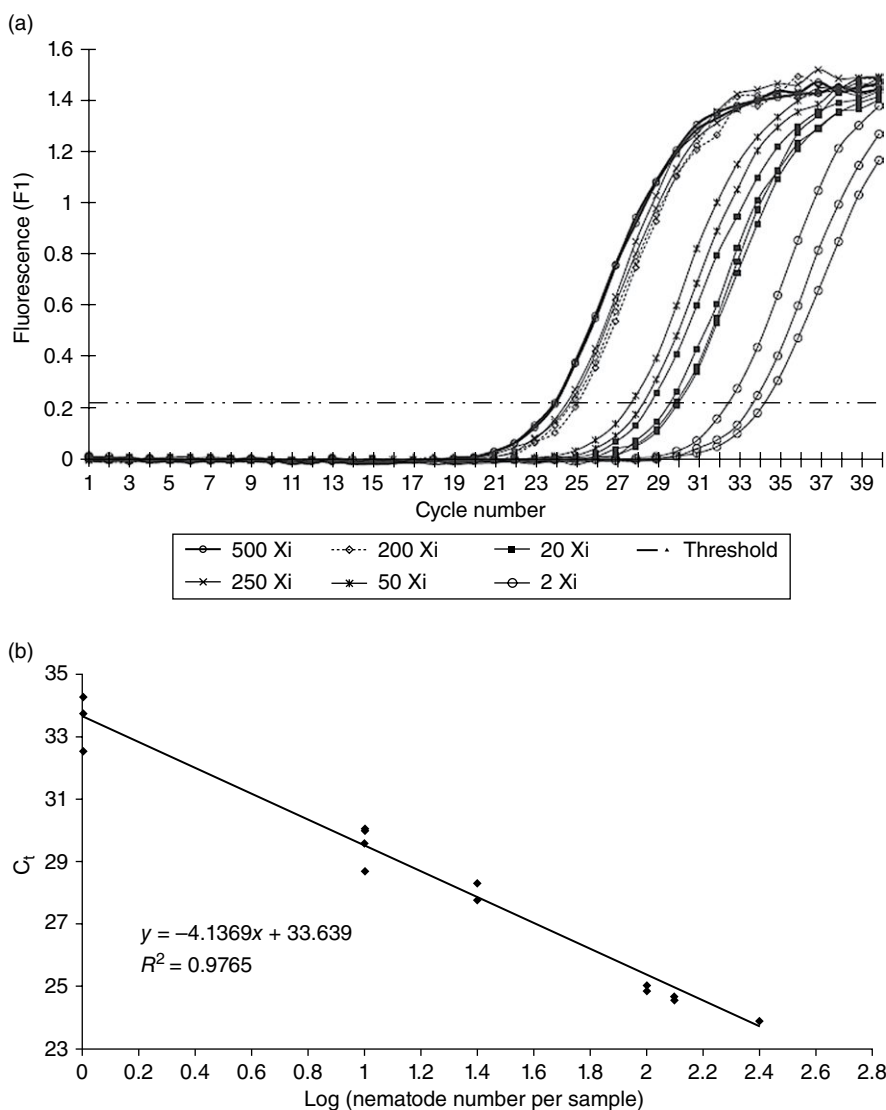


Fig. 4.9. Relationship between nematode density and the threshold cycle number (C_t) using the real-time PCR method for *Xiphinema index*. (a) Amplification curves for pure samples. From left to right, the curves correspond respectively to 500 (two replicates), 250 (two replicates), 200 (two replicates), 50 (two replicates), 20 (four replicates) and two (three replicates) individuals in a 2 μ l total volume of extraction buffer. (b) Standard linear curve of C_t plotted against the log-transformed *X. index* numbers per sample. R^2 : linear correlation coefficient. (From Van Ghelder *et al.*, 2015.)

and TaqMan assays. SYBR Green I binds only to double-stranded DNA and becomes fluorescent only when bound. This dye has the virtue of being easy to use because it has no sequence specificity and it can be used to detect any PCR product. However, the dye binds also to any non-specific product, including primer dimers, and to overcome this problem, the melting curve analysis can be employed. Increasing the temperature of the sample melts the PCR products. The non-specific product tends to melt at a much lower temperature than the longer specific product. Bates *et al.* (2002) were the first to use real-time PCR with SYBR Green I for plant parasitic nematodes, to detect *Globodera* species.

The disadvantage of using a fluorescent dye is that it binds to any double-stranded DNA and cannot be used for quantification of individual targets in a multiplex real-time PCR, because it cannot distinguish between different sequences. In this case, sequence-specific fluorescent probes, such as TaqMan probes, are needed. In the TaqMan assay, a DNA probe consisting of approximately 25–30 nucleotides in length and labelled with a fluorescent reporter permits detection only after hybridization of this probe with its complementary sequence. Cao *et al.* (2005) developed a method for detecting the pinewood nematode, *Bursaphelenchus xylophilus*, using TaqMan probes. The PCR assay detected DNA template concentrations as low as 0.01 ng. The Ct values were correlated with the DNA template concentration ($R^2 = 0.996$), indicating the validity of the assay and its potential for quantification of target DNA. The real-time PCR assay also detected DNA from single specimens of *B. xylophilus*.

Presently, real-time PCR methods have been developed for species of *Bursaphelenchus* (Kang *et al.*, 2009), *Ditylenchus* (Subbotin *et al.*, 2005), *Heterodera* (Madani *et al.*, 2005; Ye, 2012); *Globodera* (Madani *et al.*, 2005, 2008; Nowaczyk *et al.*, 2008; Nakhla *et al.*, 2010; Papayiannis *et al.*, 2013), *Meloidogyne* (Berry *et al.*, 2008; Agudelo *et al.*, 2011), *Paratrichodorus* (Holeva *et al.*, 2006), *Pratylenchus* (Sato *et al.*, 2007; Berry *et al.*, 2008; Yan *et al.*, 2012; Mokrini *et al.*, 2013), *Xiphinema* (Berry *et al.*, 2008; Van Ghelder *et al.*, 2015) (Fig. 4.9) and others.

The real-time PCR method is straightforward, sensitive and reproducible and, compared with conventional PCR methods, has several advantages. The technique allows a simultaneous

faster detection and quantification of target DNA, and the automated system overcomes the laborious process of estimating the quality of PCR product after electrophoresis.

Loop-mediated isothermal amplification (LAMP)

The LAMP technique is a simple, rapid, specific, sensitive and cost-effective nucleic acid amplification technology developed by Notomi *et al.* (2000). Amplification is completed by incubating the mixture of DNA template, a set of 4–6 specially designed primers based on six or eight distinct regions of the target DNA and a strand displacement DNA polymerase in a single tube at an isothermal temperature of 60–65°C. It provides high amplification efficiency, with replication of the original template copy, occurring 10^9 – 10^{10} times during a 15–60 min reaction. Detection of the amplification product is determined by intercalating dyes such as SYBR Green I (Fig. 4.10) or ethidium bromide, or measuring the turbidity caused by the formation of magnesium pyrophosphate. Presently, LAMP methods have been developed for *B. xylophilus* (Kikuchi *et al.*, 2009; Kanetani *et al.*, 2011), *Meloidogyne* spp. (Niu *et al.*, 2011, 2012; He *et al.*, 2013) (Fig. 4.10), *Radopholus similis* (Peng *et al.*, 2012) and *Tylenchulus semipenetrans* (Lin *et al.*, 2016). In order to identify living organisms specifically, the LAMP technique was adapted into a reverse transcriptase assay (RT-LAMP), specifically targeting RNA by isolating RNA instead of DNA and using an additional reverse transcription step before or during amplification. In order to detect living *B. xylophilus* in wood, the RT-LAMP assay was developed by Leal *et al.* (2015), detecting the presence of mRNA encoding an expansin gene. The result indicated that the RT-LAMP assay was able to detect the target expansin mRNA 2 days after the nematodes were killed, but not 4 days after their deaths. On the contrary, DNA can still be probed from nematodes even 3 months after their death.

Compared with PCR methods, the LAMP is simple to operate and does not require specialized equipment, even for the nematode extraction step, which allows for application under field conditions.

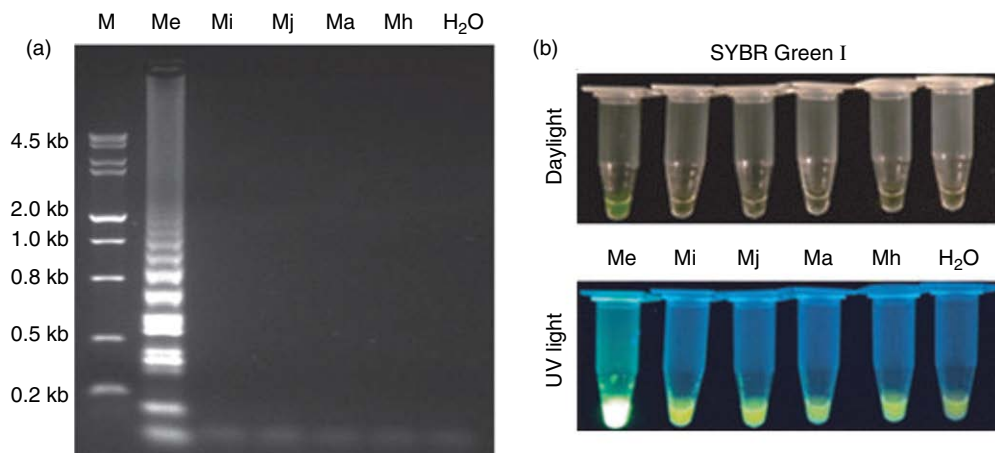


Fig. 4.10. Specificity of *Meloidogyne enterolobii* LAMP detection and product confirmation. (a) LAMP product on a gel; (b) specificity of the LAMP assay products visualized by adding SYBR Green I. Top row: direct visualization by the naked eye. Bottom row: observation under UV transillumination. M = molecular marker; Me = *M. enterolobii*; Mi = *Meloidogyne incognita*; Mj = *Meloidogyne javanica*; Ma = *Meloidogyne arenaria* and Mh = *Meloidogyne hapla*. The H₂O tube was used as a negative control without DNA template. (From Niu *et al.*, 2012.)

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5 Nematode Parasites of Rice*

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Rice (*Oryza* spp.) is the most important food crop, covering approximately 159 million hectares (Mha) in the world and producing about 700 million tonnes of paddy or 470 million tonnes of milled rice. It is the staple food for more than half of the world's population, predominantly in Asia, where more than 90% of the world's rice is grown and consumed.

Essentially, there are five major rice-growing environments (Khush, 1984), which have a profound impact on the plant parasitic nematode fauna and their concomitant damage.

1. Irrigated: about 53% of the world rice area is irrigated and provides up to 75% of the total world rice production. Irrigated (inundated) areas have good water control and rice is flooded throughout the growing season.

2. Rain-fed lowland: approximately 31% of the world rice area is planted in rain-fed lowland areas. Rain-fed lowlands have a wide variety of growing conditions related to the depth and duration of standing water on the crop. The fields are bunded, but are entirely dependent on rainfall.

3. Deep water: areas classified as deep water occur in the river deltas of South and South-east Asia, occupying about 3% of the world rice area. There is no water control, and flooding occurs only during part of the growing season when water depths vary to over 3 m.

4. Tidal wetlands: tidal wetlands occur near sea coasts and inland estuaries and are directly or indirectly influenced by tides.

5. Upland: upland rice is grown in soils without surface water accumulation. It is rain-fed without any water control. Upland rice occupies approximately 13% of the world rice area and yields are generally low. Most rice in Africa and Latin America is upland. In some areas of the world, the area under upland rice is increasing due to changes in rainfall patterns.

Nematodes of Rice

Many genera and species of plant parasitic nematodes are associated with rice, but only some of these are known or suspected to cause yield loss (Table 5.1). They have diverse parasitic habits, but all cause mechanical damage and/or malfunctions of the physiological processes involved in plant development, resulting in poor growth and yield loss. Some species cause damage in all rice environments, while others are more restricted (Table 5.1). Nevertheless, rice nematodes can be divided conveniently into two groups depending on their parasitic habits: the foliar parasites, feeding on stems, leaves and panicles; and the root parasites.

*A revision of the chapter by J. Bridge, R.A. Plowright and D. Peng in the second edition.

Table 5.1. Plant nematode genera and species known or suspected to cause yield loss in rice and their means of spread.

Nematodes	Rice affected	Means of spread
Foliar parasites		
<i>Ditylenchus angustus</i>	Lowland and deep water	Stem and panicles, soil
<i>Aphelenchoides besseyi</i>	Upland, irrigated, lowland and deep water	Seed, stem and panicles, soil
Root parasites		
<i>Criconemoides onoensis</i>	Upland, irrigated and lowland	Soil
<i>Heterodera elachista</i>	Upland and irrigated	Soil and roots
<i>Heterodera oryzae</i>	Upland and irrigated	Soil and roots
<i>Heterodera oryzicola</i>	Upland and irrigated	Soil and roots
<i>Heterodera sacchari</i>	Upland and irrigated	Soil and roots
<i>Hirschmanniella belli</i>	Irrigated, lowland and deep water	Soil and roots
<i>Hirschmanniella gracilis</i>	Irrigated, lowland and deep water	Soil and roots
<i>Hirschmanniella imamuri</i>	Irrigated, lowland and deep water	Soil and roots
<i>Hirschmanniella mexicana</i>	Irrigated, lowland and deep water	Soil and roots
<i>Hirschmanniella mucronata</i>	Irrigated, lowland and deep water	Soil and roots
<i>Hirschmanniella oryzae</i>	Irrigated, lowland and deep water	Soil and roots
<i>Hirschmanniella spinicaudata</i>	Irrigated, lowland and deep water	Soil and roots
<i>Hoplolaimus indicus</i>	Upland and irrigated	Soil and roots
<i>Meloidogyne graminicola</i>	Upland, irrigated, lowland and deep water	Soil and roots
<i>Meloidogyne hainanensis</i>	Upland and irrigated	Soil and roots
<i>Meloidogyne lini</i>	Upland and irrigated	Soil and roots
<i>Meloidogyne incognita</i>	Upland and irrigated	Soil and roots
<i>Meloidogyne javanica</i>	Upland and irrigated	Soil and roots
<i>Meloidogyne arenaria</i>	Upland and irrigated	Soil and roots
<i>Meloidogyne oryzae</i>	Irrigated	Soil and roots
<i>Meloidogyne salasi</i>	Upland and irrigated	Soil and roots
<i>Meloidogyne tritricoryzae</i>	Upland and irrigated	Soil and roots
<i>Paralongidorus australis</i>	Upland and irrigated	Soil
<i>Pratylenchus brachyurus</i>	Upland	Soil and roots
<i>Pratylenchus indicus</i>	Upland	Soil and roots
<i>Pratylenchus pseudopratensis</i>	Upland	Soil and roots
<i>Pratylenchus zaeae</i>	Upland	Soil and roots
<i>Xiphinema ifacolum</i>	Upland	Soil

Foliar Parasites

Only two species, *Ditylenchus angustus* and *Aphelenchoides besseyi*, are known foliar parasites of rice, although others are suspected.

Ditylenchus angustus

D. angustus, the cause of 'ufra' (India) or 'Tiem Dot San' (Vietnam), occurs in Bangladesh, Myanmar, India, Madagascar, Malaysia, Thailand and Vietnam, mainly in major river deltas on both deepwater and lowland rice.

Symptoms of damage

During vegetative growth, symptoms of nematode damage are prominent white patches, or white speckles in a splash pattern, at the bases of young leaves (Fig. 5.1).

Brown stains may develop on leaves and sheaths, and later intensify to a dark brown colour; leaves inside such sheaths may be wrinkled. Young leaf bases are twisted, leaf sheaths distorted, and the lower nodes can become swollen with irregular branching. After heading, infected panicles are usually crinkled, with empty, shrivelled glumes, especially at their bases; the panicle head and flag leaf are twisted and

distorted (Fig. 5.2). Panicles often remain completely enclosed within a swollen sheath, or only partially emerge (Fig. 5.3) (Butler, 1913; Hashioka, 1963; Vuong and Rabarijoela, 1968; Cox



Fig. 5.1. White patches on rice leaf base caused by *Ditylenchus angustus*. (Photograph courtesy of J. Bridge.)



Fig. 5.2. Twisting and distortion of rice panicles and flag leaf caused by *Ditylenchus angustus*. (Photograph courtesy of J. Bridge.)



Fig. 5.3. Partial emergence of a rice panicle due to *Ditylenchus angustus*. (Photograph courtesy of J. Bridge.)

and Rahman, 1980; Chakrabarti *et al.*, 1985). Dark brown patches of ultra-infected plants can be observed in the field, normally after panicle initiation (Fig. 5.4). *D. angustus* can reduce plant heights and photosynthetic rates in leaves significantly (Ali *et al.*, 1997).

Methods of diagnosis

Pieces of plant about 5 mm long are cut longitudinally to expose the innermost young leaves. Nematodes can be extracted from plant pieces placed in a small container on a Baermann funnel or small tray with water and left for 24 h or overnight before examining the suspension (see Chapter 4, this volume).

For immediate examination of material, the rolled leaves or young inflorescence can be teased apart in a Petri dish of water and observed directly. Nematodes are active in fresh material, but will require some time to resume activity from dried panicles.

Other hosts

Hosts are confined mainly to wild and cultivated species of deepwater and lowland rice (Hashioka, 1963; Vuong and Rabarijoela, 1968; Sein and Zan, 1977). Two weeds, *Echinochloa colona* and *Sacciolepis interrupta* have also been found to be infected (Cuc, 1982a).

Biology and life cycle

D. angustus is an ectoparasite, feeding on young foliar tissues. Nematodes in water invade rice within 1 h, but invasion varies with plant age, older plants being invaded less easily (Rahman and Evans, 1988). In deepwater rice seedlings, nematodes are found around the growing point, but in all parts of the plant in lowland rice. Nematodes are carried or migrate upwards to feed on newly forming tissues enclosed in the rolled leaf sheaths. They accumulate and feed on the primordia of the developing panicles; at harvest, they are coiled in a quiescent state, mainly within the dried glumes of the lower spikelets on each panicle, but not within the grains. Activity and infectivity are resumed when water returns for the next rice crop. On deepwater rice in Bangladesh, Butler (1913) assumed that multiplication of *D. angustus* took place between May/June and November, with at least three generations.



Fig. 5.4. Ufra disease. Brown patch of dead and dying rice at left caused by *Ditylenchus angustus*. (Photograph courtesy of R.A. Plowright.)

The greatest infection of rice occurs in the temperature range 27–30°C (Butler, 1913, 1919; Hashioka, 1963; Vuong and Rabarijoela, 1968; Vuong, 1969).

Survival and means of dissemination

Between crops, *D. angustus* remains active in ratoons, volunteer or wild rice (Rathaiah, 1988) and other hosts. It also survives in a desiccated state in crop residues, mainly panicles enclosed, or partially enclosed, in leaf sheaths (Cox and Rahman, 1979; Kinh, 1981b). Nematodes can be reactivated in water after 7–15 months (Butler, 1913), but may not remain infective. There is an ‘overwinter decay’ of *D. angustus* in crop residues between rice crops (Cox and Rahman, 1979), and populations decline rapidly after harvest. However, the different stages of *D. angustus* show no intrinsic ability to control water loss and survive severe desiccation. They are dependent on high humidities and/or protection by plant tissues for long-term survival (Ibrahim and Perry, 1993).

Nematodes in flooded soil are inactive in less than 4 months (Butler, 1913), and probably lose their infectivity in a much shorter period.

However, infested soil dried for 6 weeks can produce ufra disease symptoms 2 months after planting rice (Cuc, 1982b). Soil from around diseased plants does not normally appear to produce the disease (Hashioka, 1963), and is a minor component in disease transmission and nematode survival.

Most *D. angustus* die after a few days in water, but survival for longer periods has been observed (Butler, 1919). Nematode death appears to occur in water, but even a relatively brief survival in water would allow *D. angustus* to spread by water flow to infect new plants (Hashioka, 1963; Sein and Zan, 1977). Long-distance transmission in runoff water, canals and rivers is possible. Nematodes can migrate from diseased to healthy plants in water, and by stem and leaf contact under relative humidity exceeding 75% (Rahman and Evans, 1988).

D. angustus does not have an actual survival stage and cannot survive severe desiccation (Ibrahim and Perry, 1993). The nematodes can be found inside filled and unfilled spikelets of freshly harvested rice, but generally not in dried seed from infected plants (Butler, 1919; Hashioka, 1963; Sein, 1977; Cuc and Giang, 1982), apart

from one report from India (Prasad and Varaprasad, 2002), and dissemination in seed is, therefore, rare or unlikely. A small proportion of the population can enter into a facultative quiescence, and the infestation may remain in soil or crop residues for a few months.

Environmental conditions affecting parasitism

D. angustus requires at least 75% humidity to migrate on the foliage. Ufra disease is most severe in the wettest years and in the wettest areas of Bangladesh where the median rainfall exceeds 1.6 m (Cox and Rahman, 1980). In Vietnam, the disease is most severe in months of high rainfall or in fields with high water levels (Cuc and Kinh, 1981a). In the north-eastern states of India, the disease is severe in very humid and flooded areas. Flood-free years are usually ufra-free years.

Disease complexes

The ufra nematode can increase the nitrogen content of rice plants, and thus the plants become more susceptible to the plant pathogen *Pyricularia oryzae* (Mondal *et al.*, 1986). Foliar brown spots associated with the nematode could be secondary invasion sites for *Fusarium* and *Cladosporium* (Vuong, 1969).

Economic importance

Ufra has a restricted distribution because of the unique environmental requirements of the nematode. It is often localized in a rice-growing region and does not always occur in the same fields every year. In general, yield losses caused by *D. angustus* are low. In Bangladesh, for example, an annual yield loss of 4% (20% yield loss over 20% of the area) has been estimated on deepwater rice (Catling *et al.*, 1979). However, when it does occur, it is one of the most devastating of all diseases affecting rice (Cox and Rahman, 1980). *D. angustus* has been a serious problem in Vietnam in the Mekong Delta. During 1982, 60,000–100,000 ha of rice in the Mekong Delta were affected by *D. angustus* (Catling and Puckridge, 1984), and 10,000 ha in Dong Thap Province (Puckridge, 1988). Nowadays, *D. angustus* rarely causes damage, mainly because deepwater rice has been

replaced by irrigated lowland rice (Prot, 1994a). None the less, in wet seasons even lowland rice can be damaged, such as reported for Thailand (Hashioka, 1963), India (Pal, 1970; Rao *et al.*, 1986b) and Bangladesh (Mondal and Miah, 1987). Severe yield losses might occur if transplanted rice seedlings are infected with *D. angustus*, even at low initial infestation levels. Yield losses varying from 1.26 to 3.94 t/ha have been recorded with 4–10% infected seedlings (Mondal *et al.*, 1988).

Management measures

Many different measures to control *D. angustus* have been suggested; some are practical, others are less feasible. Those likely to achieve the best results are crop rotation, control of weeds and volunteer rice, control of water flow, resistant cultivars and escape cropping.

CROP ROTATION: growing a non-host crop such as jute in rotation with deepwater rice can reduce the incidence of ufra in fields where the rise of floodwater is not excessively fast (McGeachie and Rahman, 1983). Lowland transplanted rice rotated with mustard, another non-host, and jute is less affected by ufra than continuously cultivated rice (Miah and Rahman, 1985; Chakraborti, 2000).

ELIMINATING OTHER HOSTS: removal of volunteer and ratoon rice plants, wild rice and other host weeds will help prevent the carry-over of nematodes from one rice crop to the next (Hashioka, 1963; Sein and Zan, 1977).

CONTROLLING WATER FLOW: as nematodes can be spread easily in surface water, preventing river overflow into fields by improved bunding or banks could be beneficial (Sein and Zan, 1977).

RESISTANCE: several deepwater and lowland rice cultivars are known to be infected only slightly by *D. angustus*, or are even resistant (Bridge *et al.*, 2005). Resistance is mediated partly by a rapid necrotic response to nematode feeding (Plowright and Gill, 1994), and involves the increased levels of chlorogenic acid and synthesis of the rice phytoalexin sakuranetin (Plowright *et al.*, 1996). Latif *et al.* (2011) identified microsatellite markers in blast- and ufra-resistant

genotypes of rice. On the basis of these markers, parents for hybridization can be selected to develop blast- and ufra-resistant cultivars. In India and Bangladesh, the cvs. Padmapani and Digha are not attacked by *D. angustus*, suggesting that they escape the disease because of their short growth duration (Mondal and Miah, 1987; Rathaiiah and Das, 1987).

ESCAPE CROPPING: *D. angustus* survives for a limited period, and lengthening the overwinter period can reduce primary infection (Cox and Rahman, 1980; McGeachie and Rahman, 1983; Das and Bhagawati, 1992). This can be achieved with deepwater rice by using short-duration cultivars or late sowing and transplanting. Manipulation of rice cropping patterns and cultivation techniques could be a useful means of control (McGeachie and Rahman, 1983). Since *D. angustus* enters the leaf sheath primarily at the water surface (Plowright and Gill, 1994), short periods of submergence of young seedlings can reduce infection by nematodes.

CHEMICAL: chemicals have been used with some success, but their high cost makes them uneconomical.

Summary of management measures against D. angustus

The recommended management measures against *D. angustus* are broadly those put forward by the Deepwater Rice Management Project (Anon., 1987): (i) removal of crop residues to eliminate infested stem terminals; (ii) extending the overwintering period by delayed planting; and (iii) the use of shorter-duration cultivars. The use of resistant cultivars, when they become available, should prove to be the most effective measure. The burning of crop residues is no longer recommended in most countries, for environmental reasons.

Aphelenchoides besseyi

Aphelenchoides besseyi is seed borne and causes the disease 'white tip'. It has been recorded in most rice-growing areas of the world (Ou, 1985), including India, Iran, China, Italy, Turkey and Africa (Bridge *et al.*, 2005).

Symptoms

Susceptible infected plants can be symptomless, but in general, yield loss only occurs in plants showing symptoms. During early growth, the most conspicuous symptom is the emergence of the chlorotic tips of new leaves from the leaf sheath (Fig. 5.5). These tips later dry and curl, while the rest of the leaf may appear normal. The young leaves of infected tillers can be speckled with a white splash pattern, or have distinct chlorotic areas. Leaf margins may be distorted and wrinkled, but leaf sheaths are symptomless.

The viability of infected seed is lowered, germination is delayed (Tamura and Kegasawa, 1959b) and diseased plants have reduced vigour and height (Todd and Atkins, 1958). Infected panicles are shorter, with fewer spikelets and a smaller proportion of filled grain (Dastur, 1936; Yoshii, 1951; Todd and Atkins, 1958).

In severe infections, the shortened flag leaf is twisted and can prevent the complete extrusion

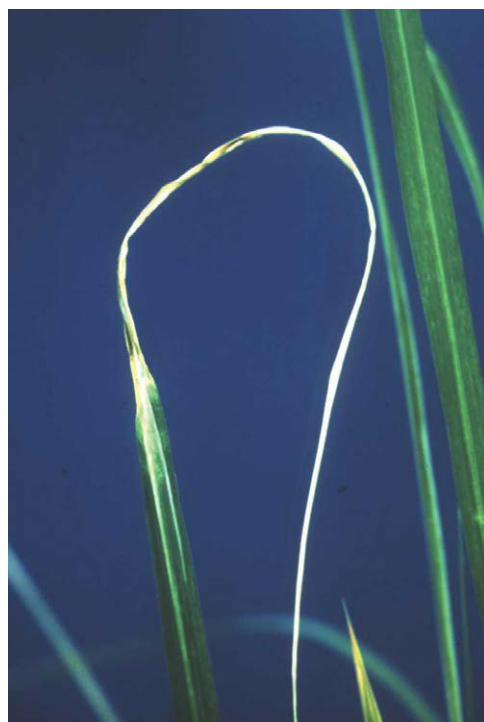


Fig. 5.5. White tip symptoms on rice leaf caused by *Aphelenchoides besseyi*. (Photograph courtesy of J. Bridge.)

of the panicle from the boot (Yoshii and Yamamoto, 1950a; Todd and Atkins, 1958). *A. besseyi* infestation reduces seed swelling (Togashi and Hoshino, 2001), the grain is small and distorted (Todd and Atkins, 1958) and the kernel may be discoloured and cracked (Uebayashi *et al.*, 1976) (Fig. 5.6). Infected plants mature late and have sterile panicles borne on tillers produced from high nodes.

A. besseyi derives its common name from its symptoms of white, hanging leaf tips of rice plants; however, in certain cultivars and conditions, no white tips may be seen and there may be only symptoms in the small grain size and erect panicles, as observed in Jiangsu, China (Wei-hong *et al.*, 2008).

Biology

When seed infected with *A. besseyi* is sown, the anabiotic nematodes rapidly become active and, during early growth, *A. besseyi* is found within the innermost leaf sheath, feeding ectoparasitically around the apical meristem (Yoshii and Yamamoto, 1950b; Goto and Fukatsu, 1952; Todd and Atkins, 1958). A rapid increase in nematode numbers takes place at late tillering (Goto and Fukatsu, 1952) and is associated with the reproductive phase of plant growth (Huang and Huang, 1972). Nematodes are able to enter spikelets before anthesis, within the boot, and feed ectoparasitically on the ovary, stamens, lodicules and embryo (Dastur, 1936; Huang and Huang, 1972). However, *A. besseyi* is more abundant on the outer surface of the glumes and enters when these separate at anthesis (Yoshii and Yamamoto, 1950b). As grain

filling and maturation proceed, reproduction of the nematode ceases, although the development of J3 to adult continues until the hard dough stage (Huang and Huang, 1972). The population of anabiotic nematodes consists predominantly of adult females (Huang *et al.*, 1979). These nematodes coil and aggregate in the glume axis. More nematodes occur in filled grain than in sterile spikelets (Yoshii and Yamamoto, 1950b), and infected grain tends to occur more towards the middle of the panicle (Goto and Fukatsu, 1952).

A. besseyi is amphimictic (Huang *et al.*, 1979), and males are usually abundant; however, reproduction can be parthenogenetic (Sudakova and Stoyakov, 1967). The optimum temperature for oviposition and hatch is 30°C. At 30°C, the life cycle is 10 ± 2 days and lengthens significantly at temperatures below 20°C (Huang *et al.*, 1972). No development occurs below 13°C (Sudakova, 1968).

Survival and dissemination

A. besseyi aggregates in the glume axis of maturing grain and slowly desiccates as kernel moisture is lost. They become anabiotic and are able to survive for 8 months to 3 years after harvest (Cralley, 1949; Yoshii and Yamamoto, 1950b; Todd, 1952; Todd and Atkins, 1958). Survival is enhanced by aggregation and a slow rate of drying (Huang and Huang, 1974), but the number (Yoshii and Yamamoto, 1950b; Sivakumar, 1987a) and infectivity (Cralley and French, 1952) of nematodes are reduced as seed age increases. It is ironic that good seed storage conditions probably prolong nematode survival. More nematodes survive in seeds stored with low moisture than in seeds at high moisture levels at most temperatures (Chaudhury and Chaudhury, 1996).

A. besseyi is not thought to survive long periods in soil between crops (Cralley and French, 1952; Yamada *et al.*, 1953), although anabiotic nematodes may survive on rice husks and plant debris. Sivakumar (1987b) found *A. besseyi* reproducing on the fungi *Curvularia* and *Fusarium* in straw after harvest.

The principal dispersal method for *A. besseyi* is seed. The inadvertent dissemination of infected seed must account for its worldwide distribution. On a local scale, *A. besseyi* can be transmitted in flood water in lowland rice (Tamura and Kegasawa,

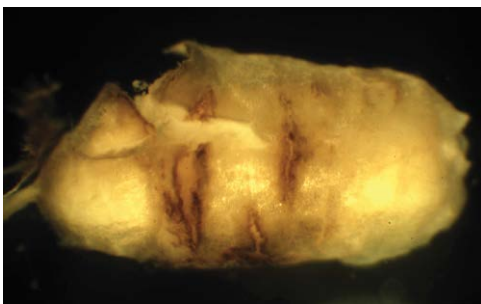


Fig. 5.6. Necrotic lesions on rice seed endosperm caused by *Aphelenchoides besseyi*. (Photograph courtesy of R.A. Plowright.)

1958), but the survival of nematodes in water decreases as temperature increases from 20 to 30°C (Tamura and Kegasawa, 1958). High seedling rates in infected seedbeds also facilitate local dispersal (Kobayashi and Sugiyama, 1977).

Environmental factors affecting parasitism

A. besseyi is able to infect rice in most environments, but infection and damage are generally greater in irrigated lowland and deepwater rice than in upland rice. In Brazil, da Silveira *et al.* (1977) found significantly more infestations in irrigated rice than in upland rice, and in Japan infection was greater in flooded conditions (Tamura and Kegasawa, 1959a).

A. besseyi is active and feeds at a relative humidity greater than 70% (Tikhonova, 1966), and consequently a high relative humidity during the reproductive phase of the crop is required for migration into the panicle (Sivakumar, 1987b) and favours symptom development (Dastur, 1936).

Other hosts

The host range encompasses more than 35 genera of higher plants (Fortuner and Orton Williams, 1975) although host races are thought to exist. The wild annual rice *Oryza breviligulata* and *Oryza glaberrima* are good hosts. Other important hosts include some common weeds of rice fields, e.g. *Cyperus iria*, *Setaria viridis* and *Panicum sanguinale* (Yoshii and Yamamoto, 1950b), and food crops such as yam, taro, sweet potato, onion, chilli pepper and maize (Hockland and Eng, 1997). In addition, many saprophytic and pathogenic fungi are also good hosts, e.g. *Alternaria* spp., *Curvularia* spp., *Fusarium* spp., *Helminthosporium* spp., *Nigrospora* sp., *Sclerotinia* sp. and *Botrytis cinerea*. Rao (1985) found that *A. besseyi* survived but did not multiply on the rice blast fungus, *P. oryzae*, and Iyatomi and Nishizawa (1954) reported that *A. besseyi* could feed and reproduce on the stem rot fungus, *Sclerotium oryzae*. *A. besseyi* could be cultured on fungi associated with rice seed and pathogenic fungi, including *Bipolaris oryzae* and *Magnaporthe solani*, but did not multiply on *Aspergillus niger*, *Rhizoctonia* and *Agaricus bisporus* (Jamali *et al.*, 2008).

Disease complexes

A. besseyi appears to influence the symptom development of some fungal pathogens of rice, such as *S. oryzae* (stem rot) and *P. oryzae* (blast) (Nishizawa, 1953a; Tikhonova and Ivanchenko, 1968; McGrawley *et al.*, 1984). *Curvularia lunata* in rice seed can cause a build-up of *A. besseyi* numbers and increases grain deformation (Rao *et al.*, 1994), and rice kernels infected by *A. besseyi* are predisposed to secondary infection by saprophytes such as *Enterobacter agglomerans*, which causes black, wedge-shaped spots on grain (Nishizawa, 1976; Uebayashi *et al.*, 1976).

Economic importance and population damage threshold levels

A. besseyi is widely distributed because of its dissemination in seed, but its importance varies between regions, countries and localities.

Damage in a susceptible cultivar depends largely on the percentage of infested seeds sown and the number of *A. besseyi* per seed. Generally, population densities per seed number or weight are counted. Fukano (1962) has determined an economic damage threshold density of 300 nematodes/100 seeds, which provides a useful basis for damage prediction since, in many countries, very little information on the pest status of *A. besseyi* exists.

Yield loss data for *A. besseyi* have been widely reported and can be as high as 45% (Bridge *et al.*, 2005). *A. besseyi* damage has been reported from deepwater rice in Bangladesh. More than 50% of fields are infected, and the panicle weight of heavily infested plants with 650 nematodes/100 seeds was one-third that of plants infected with 112 nematodes/100 seeds (Rahman and McGeachie, 1982; Rahman and Taylor, 1983). In contrast, local cultivars in Thailand appear to be tolerant of *A. besseyi* and no symptoms have been observed, despite widespread infection (Buangsuwon *et al.*, 1971). Rao (1976) has reported severe symptoms in the field in India, but accurate yield loss assessment is lacking. Muthukrishnan *et al.* (1974) observed that plants sometimes recovered after early severe damage. In Africa, *A. besseyi* is widespread, particularly in West and Central Africa, Madagascar and the Comoro Islands (Barat *et al.*, 1969).

Methods of diagnosis

Different sampling methods are used depending on the stage of crop growth (see Chapter 4, this volume). During early growth and tillering, *A. besseyi* is found in the base of the culm and between leaf sheaths. For immediate inspection, plant tissue is teased carefully in water to release nematodes. Plant tissue can be stained before examination, which is particularly useful for detecting low numbers. Alternatively, *A. besseyi* can be extracted from chopped tillers placed on a sieve, or directly in water.

During the reproductive phase, *A. besseyi* is found progressively on or in developing spikelets, and peak numbers are found at flowering. *A. besseyi* is recovered from spikelets and grain by soaking a known number in water for 24–48 h at 25–30°C. Quantitative extraction requires that the glumes are separated from the kernel yet remain in the extract. Better recovery is achieved from hulled grain, but extraction from unhulled grain is less tedious and is a practical method for detection of *A. besseyi* (e.g. for quarantine), especially if extraction time is extended to more than 2 days (Gergon and Mew, 1991).

A method that achieves very good nematode recovery is splitting individual rice seeds and then transferring into single pipette tips. Tips containing a split seed are then singly placed upright in glass vials with water (Hoshino and Togashi, 1999). However, mass extraction of split seeds to determine low levels of nematodes was as efficient and far less laborious than the single seed method (Hoshino and Togashi, 2002).

Management measures

Preventing dispersal of *A. besseyi* requires the elimination of nematodes from seed; for example, by hot water or chemical seed treatments. Resistant cultivars and cultural methods have been used to reduce infestation below damage threshold levels. Tolerant cultivars secure yield in the presence of the nematode.

HOT WATER TREATMENT: there are numerous references on the hot water treatment of rice seed (reviewed by Bridge *et al.*, 2005). The most effective control requires seed to be pre-soaked in cold water for 18–24 h, then immersed in water at 51–53°C for 15 min. Higher temperatures

(55–61°C for 10–15 min) are required if seed is not pre-soaked. The temperature and duration of treatment must be monitored closely, and after treatment, the seed must be dried at 30–35°C or sun dried if stored, but otherwise can be sown directly in the field. For quarantine purposes, at the International Rice Research Institute, seed was soaked in cold water for 3 h followed by hot water at 52–57°C for 15 min. Simply water soaking seeds followed by relatively rapid air drying can also cause marked nematode mortality in seeds (Hoshino and Togashi, 2000).

CHEMICAL: various chemical seed treatments have been used, sometimes to good effect (Ribeiro, 1977), but it is also reported that nematicide treatment of seeds has very little effect on nematode mortality within the seeds (Hoshino and Togashi, 2000). Pre-plant treatments alone are reported to be only partially effective (Aleksandrova, 1981), and there is no evidence that chemical soil treatment is an economical proposition.

RESISTANCE AND TOLERANCE: resistance to *A. besseyi* appears to be widespread. Cralley (1949) and Cralley and Adair (1949) first reported variations in the susceptibility of rice to *A. besseyi* and listed the cvs. Arkansas Fortuna, Nira 43 and Bluebonnet as resistant. In the USA, *A. besseyi* has been controlled principally through the use of resistant cultivars. Resistance to *A. besseyi* has been reported from Japan, Korea, India, Brazil, Russia and Italy (reviewed by Bridge *et al.*, 2005). Resistance to *A. besseyi* is said to be genetically controlled and carried by the Japanese cv. Asa-Hi (Nishizawa, 1953b). More recently, Wang *et al.* (2014a,b) detected several genes related to the nematode, its mortality or plant resistance in the generated transcriptome data set of *A. besseyi*. These may possibly be further explored in new resistance strategies.

CULTURAL: irrigating seedbeds (Yamada *et al.*, 1953) or direct seeding into water (Cralley, 1956) reduces infection. In these conditions, nematodes emerge and lose vigour before seed germination. High seedling rates in the seedbed (Kobayashi and Sugiyama, 1977) and high numbers of seedlings per hill (Yamada *et al.*, 1953) tend to increase infection by increasing the number of infection loci in the field. Such

problems are thought to be responsible for the reoccurrence of *A. besseyi* in Japan (Inagaki, 1985). In the USA (Cralley, 1949) and Japan (Yoshi and Yamamoto, 1951; Yamada *et al.*, 1953), early planting, presumably in cooler conditions, reduced or eliminated *A. besseyi* infection. In Korea, rotating beans with rice decreases field populations of *A. besseyi* (Kim *et al.*, 1996).

Summary of management measures against *A. besseyi*

- Hot water treatment of seed. Probably the most effective and cheapest control measure.
- Resistant or tolerant cultivars.
- Early planting if rice season is preceded by a cooler period.
- Low seedbed planting densities.

Root Parasites

Meloidogyne species

Root knot nematodes, *Meloidogyne* spp., have been found on rice in many countries. *Meloidogyne graminicola* is the main species attacking both upland (rain-fed) and lowland (irrigated) rice (Padgham *et al.*, 2004; Bridge *et al.*, 2005; Jones *et al.*, 2013, CABI, 2016). *M. graminicola* is responsible for yield failures in rice in many places in India (Gaur, 2003; Dutta *et al.*, 2012; Ravindra *et al.*, 2017).

Symptoms

All *Meloidogyne* species can cause swellings and galls throughout the root system. Infected root tips become swollen and hooked, a symptom which is especially characteristic of *M. graminicola* and *Meloidogyne oryzae* (Fig. 5.7). Galls caused by *Meloidogyne salasi* occur mostly on the root tips of rice (Sancho *et al.*, 1987). The galls of the root tip may also be club shaped. The new galls appear whitish, which turn brown or grey after 2–3 weeks. When dissected, the white galls reveal the presence of many swollen J2, J3, J4 and young females and males, while the brown or grey galls also contain several mature females, egg masses in gelatinous matrix and males. Up to 30 females occur in a typical gall on rice root produced by *Meloidogyne triticoryzae*, while 15–20 females are commonly seen in a gall of *M. graminicola* (Gaur, 2003). These multiple female galls contain several hundred eggs. The galled root does not grow in length; hence, the plant produces many lateral roots that get infected and are also galled. Thus, the root, especially in light loam or upland conditions, becomes short, stubby and excessively branched or bushy (Fig. 5.8). Elongated swollen galls may also be formed on the root far above the root tips.

For rice, most of the eggs in the gelatinous matrix remain within the root cortical aerenchyma. For wheat which lacks an aerenchyma, egg sacs of *M. graminicola* and *M. triticoryzae* extrude out of the root and become visible on the root surface. The vascular bundles are dislocated, the cortical parenchyma has hyperplasia



Fig. 5.7. Characteristic hooked root tip galls on rice caused by *Meloidogyne graminicola*. (Photograph courtesy of J. Bridge.)



Fig. 5.8. Short, stubby and excessively branched and bushy root system caused by *Meloidogyne graminicola*. (Photograph courtesy of D. Peng.)

and is extensively damaged. The root epidermis is broken, with partially protruding females and egg sacs, whereas, in rice root, female and egg sacs usually remain inside the root parenchyma (Chandel *et al.*, 2001a; Gaur, 2003).

Above-ground symptoms vary according to the type of rice and the species of *Meloidogyne*. In upland conditions and shallow, intermittently flooded land, all species can cause severe growth reduction, unfilled spikelets, reduced tillering, chlorosis, wilting and poor yield (Babatola, 1984). Symptoms often appear as patches in a field (Fig. 5.9).

M. graminicola is known to cause serious damage to deepwater rice. Prior to flooding, symptoms are the typical stunting and chlorosis of young plants. When flooding occurs, submerged plants with serious root galling are unable to elongate rapidly, and do not emerge above the water level (Bridge and Page, 1982). This causes death or drowning of the plants, leaving patches of open water in the flooded fields (Fig. 5.10).

Biology and life cycle

The biology and life cycle of *Meloidogyne incognita* and *Meloidogyne javanica* on rice are similar to those described for other crops. The life cycle of *M. oryzae* is 4 weeks at a mean temperature of 27°C (Segeren and Sanchit, 1984). *M. graminicola* from Bangladesh has a very short life cycle on rice of less than 19 days at temperatures of 22–29°C (Bridge and Page, 1982), and an isolate from the USA completed its cycle in 23–27 days at 26°C (Yik and Birchfield, 1979). In India, the life cycle of *M. graminicola* is reported to be 26–51 days, depending on the time of the year

(Rao and Israel, 1973). Females and egg masses of *M. oryzae* are completely embedded in root tissues, and up to 50 females can be present in a single gall (Segeren and Sanchit, 1984). Infective, second-stage juveniles of *M. graminicola* invade rice roots in upland conditions, just behind the root tip (Buangsuwon *et al.*, 1971; Rao and Israel, 1973). Females develop within the root, and eggs are laid mainly in the cortex (Roy, 1976a) (Fig. 5.11).

Juveniles can remain in the maternal gall or migrate intercellularly through the aerenchymatous tissues of the cortex to new feeding sites (Fig. 5.12) within the same root (Bridge and Page, 1982). This behaviour appears to be an adaptation by *M. graminicola* to flooded conditions, enabling it to continue multiplying within the host tissues even when roots are covered deeply by water. Juveniles that migrate from rice roots in flooded soil cannot reinvasion (Bridge and Page, 1982). Population growth of *M. graminicola* and *M. triticoryzae* and nematode damage to rice is enhanced by unpuddled soil and intermittent flooding conditions, providing optimum aeration for these nematodes in the soil (Garg *et al.*, 1995). On the contrary, there is less root knot invasion of rice roots when the soil is puddled (Chandel *et al.*, 2002b), and the females and egg sacs usually remain inside the root tissues (Chandel *et al.*, 2001a). *M. triticoryzae* survived for longer periods in moist and wet soil than in air-dried soil. In sterilized soil, very few J2 survived or remained infective after 45–75 days. No galls were produced after 105 days. *M. triticoryzae* J2 were very susceptible to desiccation, but the population appeared to survive in egg stage (Chandel *et al.*, 2002c).

Attraction bioassays in pluronic gel clearly showed that *M. incognita* preferred tomato roots to rice or mustard roots, while *M. graminicola* was more attracted towards rice compared with tomato or mustard roots (Dutta *et al.*, 2011). Either the blend of attractants and repellents are different in good and poor hosts or relatively long-range attractants, together with shorter-range repellents, might affect nematode movement patterns. Some host-specific attractants might also be involved. *M. incognita* was able to invade and develop to adult females, but did not produce eggs in rice roots. By contrast, *M. graminicola* developed and reproduced faster than *M. incognita* on both rice and tomato plants.



Fig. 5.9. Typical patches showing severe growth reduction and chlorosis in intermittently flooded rice in China. (Photograph courtesy of D.L. Peng.)



Fig. 5.10. Drowning out of rice plants due to stunting of seedlings before flooding by *Meloidogyne graminicola* in Bangladesh. (Photograph courtesy of J. Bridge.)

Nevertheless, second-stage juveniles of both these root knot nematodes inside the roots accumulate at the root tips of rice. Molecular analysis of the root exudates indicated the presence of

certain semiochemicals, including small lipophilic molecules that differed between those two plants, and might explain the differences in attraction and parasitism (Dutta *et al.*, 2012).

Patil *et al.* (2013) found that the J2 of *M. graminicola* were attracted towards the roots of rice plants grown in hydroponics containing 0.1 mM NO_3^- and 2.85 mM $\text{Ca}(\text{NO}_3)_2$, but repelled by 10.0 mM NO_3^- , 2.85 mM NH_4NO_3 and NH_4Cl . The results suggest that the application of ammonia-based nitrogen fertilizer to the rice nursery bed may interfere with nematode attraction and thus reduce invasion, and the application of chemical nitrification inhibitors to rice nursery beds may decrease nematode invasion.

Biological races

Rice cultivars are susceptible to race 1 of *Meloidogyne arenaria* and races 2 and 4 of



Fig. 5.11. Stained females and eggs of *Meloidogyne graminicola* within massive gall on rice root. (Photograph courtesy of J. Bridge.)

M. incognita (Ibrahim *et al.*, 1983). Significant variations in the infectivity of *M. triticroyzae* and four geographically different populations of *M. graminicola* against 27 selected crops and weeds have been observed (Sabir and Gaur, 2005).

Survival and means of dissemination

M. incognita, *M. javanica*, *M. arenaria* and *M. salasi* are parasites mainly of upland rice and survive in soil as eggs or juveniles, or on alternative hosts. They do not survive long periods in flooded soil. *M. oryzae* can survive in shallow flooded (<10 cm) rice fields for relatively short periods (Segeren and Sanchit, 1984), but *M. graminicola* is well adapted to flooded conditions and can survive in waterlogged soil as eggs in egg masses or as juveniles for long periods. Numbers of *M. graminicola* decline rapidly after 4 months, but some egg masses can remain viable for at least 14 months in waterlogged soil (Roy, 1982). *M. graminicola* can survive in soil flooded to a depth of 1 m for at least 5 months (Bridge and Page, 1982); it cannot invade rice in flooded conditions but quickly invades when infested soils are drained (Manser, 1968). All *Meloidogyne* spp. can be spread in soil and on seedlings of other crop hosts planted in a field. Because *M. oryzae* and, especially, *M. graminicola* are found in flooded rice, there is the additional danger of dissemination in irrigation and run-off water.



Fig. 5.12. Histological section through *Meloidogyne graminicola* galled root tissue showing feeding site with giant cells (right) and adult female (left). (Photograph courtesy of J. Bridge.)

Population dynamics

The population density of *M. graminicola* J2 in soil varies greatly over the year, depending on prevailing temperature, moisture and plant-growth stage. In north India, peak numbers of J2 of *M. graminicola* and *M. triticorniae* in soil occurred in September/October, coinciding with the earhead emergence and grain formation of the rice crop; a smaller peak was observed during March, when the wheat crop was in milky grain stage (Gaur and Singh, 1997; Gaur, 2003). This is the time when soil is not flooded but moist, soil temperature is moderate (25–30°C) and the older galled roots begin to disintegrate and release hatched J2. In general, 3–4 generations are completed in rice and 1–2 in wheat.

In the Philippines, *M. graminicola* was the most prevalent and abundant plant parasitic nematode. *Hirschmanniella* was the second most prevalent genus in lowland rice, but its density was low in upland rice (Pascual *et al.*, 2014). In Bangladesh, the root population of *M. graminicola* showed two distinct peaks – at the maximum tillering stage of the rice plants in January and at the heading stage of the rice plants in March (Win *et al.*, 2013). Root galling caused by *M. graminicola* followed the same trend as the J2 population densities throughout the irrigated season.

Gaur *et al.* (2000) found that *M. triticorniae* produced three kinds of unhatched J2: (i) those that hatched freely in water; (ii) those that required stimulus from rice root diffusate; and (iii) those that did not hatch even in the presence of diffusate. The proportion of these three types varied throughout the cropping cycle, with the final generation produced on senescing plants having a large proportion of unhatched J2 of the third type, which was likely to equate with diapause. Dilution of the diffusate reduced its hatching activity and there was no evidence of the presence of inhibitors in the diffusate. The hatching response of *M. triticorniae* may be modified additionally by the growing conditions: submergence of infected plants may have delayed hatch, possibly by causing anoxia in unhatched J2, and thereby delaying the appearance of the second and third generations of the nematodes. *M. triticorniae* survived for longer periods in moist and wet soil

than in air-dried soil. In sterilized soils, very few second-stage juveniles survived or remained infective after 45–75 days. No galls were produced after 105 days. *M. triticorniae* J2 were very susceptible to desiccation but the population appeared to survive in egg stage (Chandel *et al.*, 2001b).

Alternative hosts of Meloidogyne

M. incognita, *M. javanica* and *M. arenaria*, the most widespread root knot species, have numerous hosts other than rice. *M. graminicola* also has a wide host range (CABI, 2016) that includes many of the common weeds of rice fields (Table 5.2). It is parasitic on both the *indica* and *japonica* races of *Oryza sativa* (Manser, 1971), and can also be a damaging parasite of vegetables, such as onion (Gergon *et al.*, 2001). A number of weeds and crops are also alternative hosts of *M. oryzae* (Maas *et al.*, 1978; Segeren and Sanchit, 1984) and *M. salasi* (Lopez, 1984; Salazar and Quesada, 1999).

M. triticorniae in India is known to reproduce on wheat, barley, sorghum, soybean, okra, green gram, berseem (*Trifolium alexandrinum*) and some cultivars of potato, as well as on the weed species, *Cyperus rotundus*, *Echinochloa colonum*, *Echinochloa crus-galli*, *Leptochloa coloniculus* and *Phalaris minor* (Gaur and Sharma, 1998; Chandel *et al.*, 2002a). In some areas in north India where rice cultivation has recently been introduced, the early appearance of severe damage to rice by root knot nematodes could be attributed to the fact that the sorghum and millets traditionally grown there for fodder would have supported these nematodes (Gaur, unpublished observation).

Economic importance

M. graminicola can cause economic yield loss in upland, lowland and deepwater rice. In upland rice, there is an estimated reduction of 2.6% in grain yield for every 1000 nematodes present around young seedlings (Rao and Biswas, 1973). The population levels that cause 10% loss in yield of upland rice are 120, 250 and 600 eggs/plant at 10, 30 and 60 days age of plants, respectively, in direct seeded crops (Rao *et al.*, 1986a). In flooded rice, damage by *M. graminicola* is caused in nurseries before transplanting

Table 5.2. Hosts of *Meloidogyne graminicola*. (From: Birchfield, 1965; Buangsuwon et al., 1971; Manser, 1971; Roy, 1977; Yik and Birchfield, 1979; MacGowan and Langdon, 1989.)

<i>Abelmoschus esculentus</i>	<i>Impatiens balsamina</i>
<i>Ageratum conyzoides</i>	<i>Imperata cylindrica</i>
<i>Allium cepa</i>	<i>Lactuca sativa</i>
<i>Alopecurus carolinianus</i>	<i>Leersia hexandra</i>
<i>Amaranthus viridis</i>	<i>Leucas lavendulaefolia</i>
<i>Ammania petandra</i>	<i>Ludwigia repens</i>
<i>Andropogon</i> sp.	<i>Lycopersicon esculentum</i>
<i>Avena sativa</i>	<i>Murdannia nudiflora</i>
<i>Beta vulgaris</i>	<i>Musa</i> sp.
<i>Blumea</i> sp.	<i>Oplismenus compositus</i>
<i>Borreria hispida</i>	<i>Oryza sativa</i>
<i>Borreria ramosa</i>	<i>Oxalis corniculata</i>
<i>Brassica juncea</i>	<i>Panicum miliare</i>
<i>Brassica oleracea</i>	<i>Panicum miliaceum</i>
<i>Catharanthus roseus</i>	<i>Panicum repens</i>
<i>Centella asiatica</i>	<i>Paspalum scrobiculatum</i>
<i>Commelina benghalensis</i>	<i>Pennisetum typhoides</i>
<i>Colocasia esculenta</i>	<i>Pennisetum pedicellatum</i>
<i>Corchorus capsularis</i>	<i>Petunia</i> sp.
<i>Courtosia cyperoides</i>	<i>Phaseolus vulgaris</i>
<i>Cucumis sativus</i>	<i>Phlox drummondii</i>
<i>Cymbopogon citratus</i>	<i>Phyllanthus urinaria</i>
<i>Cynodon dactylon</i>	<i>Pisum sativum</i>
<i>Cyperus brevifolius</i>	<i>Poa annua</i>
<i>Cyperus compressus</i>	<i>Portulaca oleracea</i>
<i>Cyperus deformis</i>	<i>Ranunculus pusillus</i>
<i>Cyperus iria</i>	<i>Rungia parviflora</i>
<i>Cyperus pilosus</i>	<i>Saccharum officinarum</i>
<i>Cyperus procerus</i>	<i>Sacciolepis indica</i>
<i>Cyperus pulcherrimus</i>	<i>Scirpus articulatus</i>
<i>Cyperus rotundus</i>	<i>Scoparia dulcis</i>
<i>Desmodium triflorum</i>	<i>Setaria italica</i>
<i>Digitaria longiflora</i>	<i>Solanum melongena</i>
<i>Digitaria sanguinalis</i>	<i>Solanum nigrum</i>
<i>Echinochloa colonum</i>	<i>Solanum sisymbriifolium</i>
<i>Echinochloa crusgalli</i>	<i>Sorghum bicolor</i>
<i>Eclipta prostrata</i>	<i>Sphaeranthus africanus</i>
<i>Eleusine coracana</i>	<i>Sphenoclea zeylanica</i>
<i>Eleusine indica</i>	<i>Spinacea oleracea</i>
<i>Eragrostis gangetica</i>	<i>Stellaria media</i>
<i>Eragrostis plumosa</i>	<i>Trifolium repens</i>
<i>Euphorbia hirta</i>	<i>Triticum aestivum</i>
<i>Fimbristylis miliacea</i>	<i>Urena lobata</i>
<i>Fimbristylis podocarpa</i>	<i>Vandellia</i> sp.
<i>Fuirena glomerata</i>	<i>Vernonia cinerea</i>
<i>Glycine max</i>	<i>Vicia faba</i>
<i>Gnaphalium purpureum</i>	<i>Vigna mungo</i>
<i>Grangea maderaspatana</i>	<i>Vigna radiata</i>
<i>Hedyotis diffusa</i>	<i>Vigna unguiculata</i>
<i>Herminium</i> sp.	<i>Zea mays</i>

(Fig. 5.13); the tolerance limit of seedlings is less than one second-stage juvenile/cm³ of soil (Plowright and Bridge, 1990). Damage also

occurs prior to flooding where rice is sown directly in well-drained soils. Experiments have shown that 4000 juveniles/plant of *M. graminicola*



Fig. 5.13. Newly germinated rice seedling severely galled by *Meloidogyne graminicola*. (Photograph courtesy of D.L. Peng.)

can cause destruction of up to 72% of deepwater rice plants by drowning out. Losses as high as this in the field are unlikely, as natural root populations vary considerably (Bridge and Page, 1982).

M. incognita can cause poor seedling establishment and reduced yields in upland rice. Yields can decrease to 60% when 8000 eggs and juveniles/100 ml soil are present at sowing (Babatola, 1984). Significant yield reductions can occur in both upland and irrigated rice with *M. incognita* (Ibrahim *et al.*, 1972), but damage is generally more severe under upland conditions (Fademi, 1984). Damage to irrigated rice will occur where seedlings are raised in well-drained nursery soils. High initial soil populations of both *M. incognita* and *M. javanica* are necessary to cause yield loss in rice, and populations above 1000 eggs/plant are needed to reduce grain yield with *M. javanica* (Sharma, 1980) or as high as 35,000 eggs/plant to reduce growth by around 40% (Ferraz, 1995). Populations of 128 eggs and juveniles/cm³ of Venezuelan isolates of *M. incognita* have been shown to kill rice plants (Greco *et al.*, 2000).

Generally, crop damage has been measured in terms of loss of grain yield. Losses due to poor quality of the grain to be used as food or seed have received little attention. Nie *et al.* (2009) reported an increase in 1000 grain weight of rice in the crop grown after two seasons of fallow or three seasons of flooded rice. Patil and Gaur (2014) reported that population densities of 8 J2/g soil of *M. graminicola* reduced the 1000 grain weight by as much as 44.5% in cv. Pusa Sugandh-5 and 50.7% in cv. Pusa-44. The

protein and amylose contents of the grains were reduced significantly in both the rice cultivars. The seed germination percentages of both rice cultivars were also reduced as the nematode inoculation rates increased from 1 to 8 J2/cm³ in pot and field experiments. There also was a significant decrease in the seedling vigour index. Thus, the rice grains produced on plants infected with *M. graminicola* were lighter in weight and had poorer nutrient qualities, such as amylose and protein content. Furthermore, when these grains were used as seed, the germination rate was lower and, even more importantly, seed vigour was poor compared to seeds obtained from plants grown in non-infested soil.

Molecular method of diagnosis

Molecular methods for species identification are covered in Chapter 4 of this volume. Species-specific primers are now available for all relevant root knot nematode species including *M. graminicola* (Htay *et al.*, 2016) and can be used in single or multiplex polymerase chain reaction (PCR) tests. Internal transcribed spacer (ITS)-ribosomal deoxyribose nucleic acid (rDNA) analysis of individual juveniles of *M. graminicola* from different locations in Myanmar and China showed a low level of genetic variation among the isolates; however, all populations could be clearly separated (Htay *et al.*, 2016).

Management measures

In general, no single strategy for the management of migratory and endoparasitic nematodes can suffice. The control options have to be tailored to the nematode species, its life cycle and survival adaptations, in relation to crop type, location and economic returns (Sethi and Gaur, 1986; Moens and Perry, 2009).

The recommended control of *Meloidogyne* on rice depends on the species. Flooding of soil, even for relatively short periods, will control *M. incognita*, *M. javanica* and *M. arenaria*, and probably *M. salasi*, but continuous flooding would be necessary for *M. oryzae* and *M. graminicola*. Increasing soil fertility can compensate for some damage by the nematodes (Diomandé, 1984). If available, resistant cultivars are the most effective and economic control option, and some resistance to different species has been found (see below).

Chemical control on the field scale is generally uneconomical, particularly with low-yielding upland rice, but could be an economical proposition for nursery soils.

FLOODING: *M. incognita*, *M. javanica* and *M. arenaria* are less important parasites of lowland rice, except in seedling nurseries, and can be controlled by flooding where this is possible. Although *M. oryzae* can survive some flooding, it can be controlled at depths greater than 10 cm (Segeren and Sanchit, 1984). It is mainly a problem in the elevated areas of flooded rice fields where levelling is poor. *M. graminicola* will survive normal flooding, but damage to the crop can be avoided by raising rice seedlings in flooded soils, thus preventing root invasion by the nematodes (Bridge and Page, 1982). Continuous flooding is highly effective in controlling *M. graminicola* in Vietnam (Kinh *et al.*, 1982). Similarly, in the Philippines, yield losses due to *M. graminicola* may be prevented or minimized when the rice crop is flooded early and kept flooded until a late stage of development (Soriano *et al.*, 2000).

There has been an increase in the incidence of *M. graminicola* damage in rice fields in the Philippines related to a decrease in the availability of water for agricultural use, and the nematode is found mainly in non-permanently flooded fields (Prot, 1994a; Prot *et al.*, 1994). Flooding the soil for 3 weeks prior to transplanting to control weeds has often been replaced with the more economical use of direct wet seeding in saturated but non-flooded soils. Also, farmers are more likely to use intermittent rather than continuous flooding, to save water. Both of these water management activities allow juveniles of *M. graminicola* to parasitize roots from the soil, which they are unable to do in continuously flooded conditions (Prot, 1994a).

Puddling of soil reduces the bulk density of soil and decreases the hydraulic conductivity in the upper layers, but not in the deeper layers of soil. Aeration is reduced due to high moisture levels retained in the puddled soil. Puddling of soil prior to transplanting and prolonged early flooding reduces populations of both *M. graminicola* and *M. triticoryzae* in rice fields in India (Garg *et al.*, 1995). However, if the seedlings were already infected before transplanting and submergence, the nematode could survive well

and reproduce within the aerenchyma of the root (Chandel *et al.*, 2002c).

Continuous cultivation of aerobic rice caused yield decline. Two seasons of fallow or three seasons of flooded rice resulted in significantly higher biomass, spikelets per m², 1000 grain weight and higher yields than continuous aerobic rice (Nie *et al.*, 2009).

CONSERVATION AGRICULTURE PRACTICES: a number of resource conservation techniques, including zero tillage, crop residue incorporation, direct seeding of rice, raised-bed cultivation of rice, system of rice intensification (SRI), etc., have been advocated by agronomists during the past decade. Nematologists have reported varying effects on nematode population densities and crop damage. In general, higher population densities of root knot nematodes in rice have been reported under zero or reduced tillage, SRI, raised-bed, direct seeded or aerobic rice, reduced or intermittent irrigation, etc. The incorporation of organic matter into soil in the form of crop residues or organic amendments reduced these nematodes (Gaur and Singh, 1993; Pankaj *et al.*, 2006; Upadhyay *et al.*, 2014).

SOWING TIME OF WINTER CROP: in the rice–wheat cropping system, the rice crop intervening the two wheat crops affects the population density of the nematode for the following rice crop. Although wheat is a very good host of both *M. graminicola* and *M. triticoryzae*, the low temperature (<15°C) prevailing during the winter wheat season suppresses the activity and reproduction of those root knot nematodes. In areas with cool winters, delaying the sowing of wheat could help reduce population growth of root knot nematodes for the subsequent rice crop. The infection and population growth of *M. triticoryzae* (Gaur and Sharma, 2000) and *M. graminicola* (Singh *et al.*, 2003) was less on the wheat crop sown in late November to mid-December, when temperature is low, compared to the early sown crop. Solarization of the nursery beds under 40–50 µm clear polyethylene sheet for 3 weeks and the application of carbosulfan 3G at 3.3 g/m² prior to sowing rice seed helped in producing nematode-free rice seedlings that, after transplanting, established better and gave a healthier rice crop (Chandel *et al.*, 2002c).

RESISTANCE: a number of rice cultivars and breeding lines have been recorded as resistant to *Meloidogyne* species, although only a small number of these are truly resistant. Diomandé (1984) found that cultivars of *O. glaberrima* were resistant to *M. incognita*. Generally, cultivars of *O. sativa* were susceptible, although some improved cultivars, such as IRAT 109, IRAT 112, IRAT 133, IRAT 106, and a traditional cultivar, CG-18, showed tolerance. Rice cvs. IR 28, IR 459 and P24 are resistant to *M. arenaria*, *M. javanica* and *M. incognita* (races 2, 3 and 4), and A95; Giza 171 and Giza 172 are resistant to *M. incognita* (race 3) and *M. javanica* (Ibrahim *et al.*, 1983). The cvs. IR 20, Ikong Pao Faro 21 and 27 support low populations of *M. incognita* in Nigeria (Babatola, 1980; Fademi, 1987). The majority of *O. sativa* cultivars are susceptible to *M. graminicola*. For example, all 80 cultivars tested in Laos were found to be susceptible (Manser, 1971). However, there are a number of cultivars from India, Thailand and the USA that are reported to be resistant to *M. graminicola* (Roy, 1973; Jena and Rao, 1974, 1976; Prasad *et al.*, 1979, 1986b; Yik and Birchfield, 1979; Chunram, 1981; Rao *et al.*, 1986b). It has been shown that tolerance levels of rice cultivars to *M. graminicola* are affected by whether the crop is grown in upland or flooded conditions (Tandingan *et al.*, 1996). Resistance against *M. graminicola* was found in certain cultivars of the low-yielding *O. glaberrima* that could be transferred to higher-yielding *O. sativa* by cross breeding (Plowright *et al.*, 1999; Bimpong *et al.*, 2010). Attempts have been made to understand the mode of resistance. Lilley *et al.* (2011b) described the delivery of a nematode-repellent peptide using a root-cap-specific promoter.

Cabasan *et al.* (2012) compared the penetration, development and reproduction of *M. graminicola* on four resistant rice cultivars from Africa and two susceptible cultivars from Asia. Although attraction was similar on both sets of cultivars, penetration, development and reproduction was reduced on the African cultivars. The early hypersensitive defence response led to the collapse or degeneration of the gall before females could develop. The resistant cultivars of *O. glaberrima* have lesser root and stele diameter and cortical thickness compared to the susceptible *O. sativa* cultivars (Cabasan *et al.*, 2013).

Our continuously increasing knowledge about resistance mechanisms and the enormous potential of modern molecular techniques such as RNA interference or CRISPR (clustered regularly interspaced short palindromic repeats) will improve breeding efforts for resistance in the future (Lilley *et al.*, 2011a, 2012).

A lot of effort has been made to breed cultivars resistant to abiotic stresses like drought, salinity, temperature and biotic resistance against an array of pests and pathogens. Asselbergh *et al.* (2008) studied the reaction of the plant to biotic and abiotic stresses. They found that an increase in abscisic acid led to increased tolerance to abiotic stresses like drought, cold and salinity, but decreased the tolerance to biotic stresses, including root knot nematodes. A high level of abscisic acid was found to suppress the plant stress hormones jasmonic acid, ethylene and salicylic acid, leading to increased susceptibility to root knot nematodes. On the contrary, low levels of abscisic acid resulted in increased resistance to pathogens.

CROP ROTATION: certain crops are resistant or poor hosts of *M. graminicola* and could be used in rotation, to reduce nematode populations, e.g. castor, cowpea, sweet potato, soybean, sunflower, sesame, onion, turnip, common bean, jute and okra (Rao *et al.*, 1986a). Soil populations of *M. graminicola* are reduced when rice is preceded by the planting of mustard (*Brassica campestris* subsp. *oleifera*) and guizil (*Guizotia abyssinica*) in Bangladesh (Rahman, 1990). Rotations longer than 12 months will be needed to reduce *M. graminicola* soil populations. Introducing a fallow into the rotation will also give control of the nematodes but, to be effective, needs to be a bare fallow free of weed hosts (Roy, 1978), and is therefore impractical in most circumstances. However, one weed, *Eclipta alba*, is toxic to *M. graminicola* and could be grown and incorporated into the field to kill the nematodes (Prasad and Rao, 1979b). In the rice–wheat cropping system prevalent in north India, the replacement of wheat with mustard, mungbean or lentil between two rice crops could reduce the population density of *M. graminicola* for the next rice crop (Chandel *et al.*, 2002a). Growing a short-duration crop of mungbean during the summer intervening wheat and rice crops and the incorporation of the green stover after

harvest of the pods also significantly reduced the root knot nematode infection and damage to rice (Chandel *et al.*, 2002a; Gaur, 2003).

SOIL AMENDMENTS: the use of decaffeinated tea waste and water hyacinth compost has been suggested to control *M. graminicola* (Roy, 1976b), and some reduction in populations is reported following the incorporation of other chopped 'botanicals', *Polygonum*, *Ageratum*, *Mikania* and water hyacinth (*Eichhornia crassipes*) (Das *et al.*, 1999). The application of organic amendments like biomass of sesame, buckwheat, neem leaves, chinaberry leaves or chicken manure to soil reduced the infection and damage to rice by *M. graminicola* (Dangal *et al.*, 2008). The form of nitrogen supply to the plant could also influence the nematode infection and susceptibility of the plant. Patil *et al.* (2013) found rice plants supplied with nitrogen in nitrate form were infected more by *M. graminicola* than plants receiving an equal amount of N in ammonical form. This might also explain why aerobic rice, with soil having nitrate abundance, suffers more damage than irrigated rice where the ammonical form is more abundant. There are many reports of greater root knot nematode damage to dry-bed, aerobic or intermittently irrigated rice than the wet-bed or continuously irrigated rice (Gaur and Singh, 1993, 1997; Chandel *et al.*, 2002a,b; Dangal *et al.*, 2009, 2010; Pankaj *et al.*, 2010). Yield failure of aerobic rice occurred in the Philippines due to *M. graminicola* (Kreye *et al.*, 2009a). Differences were noted in the effects of irrigation after 1 or 2 weeks. The nematode multiplied more under irregular irrigation regimes (Kreye *et al.*, 2009b).

BIOLOGICAL CONTROL: *Trichoderma harzianum*, *Trichoderma virens* and *Trichoderma viride* have been reported to suppress plant parasitic nematodes, including *M. graminicola* (Pathak and Kumar, 1995, 2003), and therefore may help in the management of root knot nematodes under aerobic rice cultivation. The endophytic bacterium *Bacillus megaterium*, isolated from the roots of lowland rice in Taiwan, reduced the attraction of *M. graminicola* to rice roots and decreased juvenile hatch from eggs (Padgham and Sikora, 2007). Le *et al.* (2009) inoculated roots of rice seedlings with conidia of endophytic and rhizosphere colonizing *Fusarium* isolates and observed reduced root galling by 29–42%

and increased root weight by up to 33%. *Trichoderma* species, which were recovered from only the rhizosphere of the rice plants, showed similar biological control effects on nematode galling and on plant growth by reducing galling severity up to 38%. Priya (2015), attempting biocontrol of *M. graminicola* in aerobic rice, found that *T. viride* was more effective than *Purpureocillium lilacinum*, *Pseudomonas fluorescens* and *Bacillus subtilis*. Furthermore, *Pythium arhenomanes* has been shown to influence *M. graminicola* in the rice root, resulting in improved plant growth and yield, especially at high pathogen pressure (Verbeek *et al.*, 2016). The predatory fungus *Dactylaria brochopaga* was found to suppress *M. graminicola* on wheat (Kumar and Singh, 2011), while the vesicular arbuscular fungi *Glomus mossae*, *Glomus fasciculatum* and *Gigaspora* sp. increased the tolerance of onions to *M. graminicola* and improved plant growth. Onion and wheat are grown between two rice crop seasons in some places and are both hosts of *M. graminicola*. Le *et al.* (2009, 2016) demonstrated that seed treatment with the mutualistic endophyte *Fusarium moniliforme* Fe14 reduced infection by up to 52%, as well as reproduction. More significantly, the presence of the endophyte in the root caused a nine-fold increase in the male to female ratio. It should be noted that, to date, there are no bio-control agents used on a practical field basis.

SOIL SOLARIZATION: the method can be effective on a small scale, such as on nursery beds. It uses clear polyethylene sheets, which are laid on the surface of the beds for a period of 3–4 weeks in sunny weather. It can give a reduction of populations of *Meloidogyne* spp. in rice beds of over 80% and improve seedling growth (Gaur and Perry, 1991; Ganguly *et al.*, 1996; Chandel *et al.*, 2002d; Gaur, 2003). Solarization requires intense solar energy, otherwise the heat produced is either sublethal or it only controls the nematodes in the very upper reaches of the soil (see Chapter 23, this volume).

CHEMICALS: seed treatments, root dips, soil drenches and soil incorporation have been tested in experimental trials, with varying success in India (Rao *et al.*, 1986a; Rahman and Das, 1994), but their practical and economic applicability have not been determined.

INTEGRATED NEMATODE MANAGEMENT: based on research over the preceding decade, Gaur (2003) developed an integrated nematode management package for the rice–wheat cropping system prevalent in the scented (basmati) rice in north India. This package suppressed the population growth of *M. graminicola* and *M. triticoryzae* and increased grain yield by 12% in moderately infested fields:

1. Nursery bed. Nursery beds should be established at a site where no rice has been grown during the previous 2 years. (i) Soil solarization of the area marked for nursery beds using colourless, thin (25–60 µm) polythene sheet mulch for 3 weeks in May. (ii) Application of nematicides registered for soil application mixed within the upper 5 cm of soil at the time of sowing.
2. Main field. (i) Ploughing twice at 15-day intervals with a soil turning harrow, in May to June. (ii) Good puddling before transplanting. (iii) Use of healthy, nematode-free seedlings from treated nursery beds. (iv) Late sowing of wheat in late November to mid-December using a suitable late-sown cultivar. (v) In case of heavy infestation, replacing wheat with mustard in winter.

Diagnosis

The presence of *Meloidogyne* in rice roots can be determined by standard root-staining techniques or by the occurrence of galls (see Chapter 4, this volume). Root extractions will only isolate hatched juveniles and males, and a combination of root maceration and staining of a known weight of roots can be a more efficient and practical way of determining populations of sedentary females within roots. Assessing the severity of root damage by grading of root galling is a practical method, but can be difficult with rice. A rice root knot rating chart has been devised making use of the actual percentage of roots galled to determine the extent of damage caused by *M. graminicola* (Bridge *et al.*, 2005) (Fig. 5.14). Short roots and excessive branching of roots may be seen with most new roots galled. Since the egg masses remain inside the root tissue, the root pieces, and especially the hooked or clubbed root tips, lie in the dry soil and serve as reservoirs in which the unhatched eggs survive. When extraction is attempted using traditional sedimentation and sieving techniques, these root pieces

get removed and the root knot nematodes are not detected. Hence, during the periods when the hatched second-stage juveniles are not present in soil, the examination of soil samples fails to detect the presence of *M. graminicola* and *M. triticoryzae* even when the field is infested. Bioassay by using rice or wheat seeds can be very effective in detection and assessment of the severity of infestation (Gaur and Sharma, 1999).

Hirschmanniella

A number of *Hirschmanniella* species, known collectively as rice root nematodes, are parasites of irrigated, lowland and deepwater rice (Table 5.1). They are found in flooded fields and occur in the majority of rice-growing regions. Seven species are reported to damage rice (*Hirschmanniella belli*, *Hirschmanniella gracilis*, *Hirschmanniella imamuri*, *Hirschmanniella mexicana* (= *Hirschmanniella caudacrena*), *Hirschmanniella mucronata*, *Hirschmanniella oryzae* and *Hirschmanniella spinicaudata*) (Table 5.1), while a further 12 species have been found in rice roots (*Hirschmanniella diversa*, *Hirschmanniella dubia*, *Hirschmanniella indica*, *Hirschmanniella kaverii*, *Hirschmanniella magna*, *Hirschmanniella mangalorensis*, *Hirschmanniella marina*, *Hirschmanniella microtyla*, *Hirschmanniella nghetinhensis*, *Hirschmanniella ornata*, *Hirschmanniella shamimi* and *Hirschmanniella thornei*). Seventeen species are known from rice in China (Wang and Pan, 1999; Liao *et al.*, 2000) and four species have been recorded from weeds in rice fields (*Hirschmanniella asteromucronata*, *Hirschmanniella furcata*, *Hirschmanniella obesa* and *Hirschmanniella truncata*).

Symptoms of damage

There are no easily identifiable above-ground symptoms of nematode damage in the field. Retardation of plant growth occurs especially in early stages; also a decrease in tillering. Yellowing of rice plants is observed occasionally (Fig. 5.15), and flowering can be delayed by up to 14 days. Roots invaded by *Hirschmanniella* spp. turn yellowish brown and rot.

Biology

Hirschmanniella spp. are migratory endoparasites of roots (Fig. 5.16). The nematodes produce

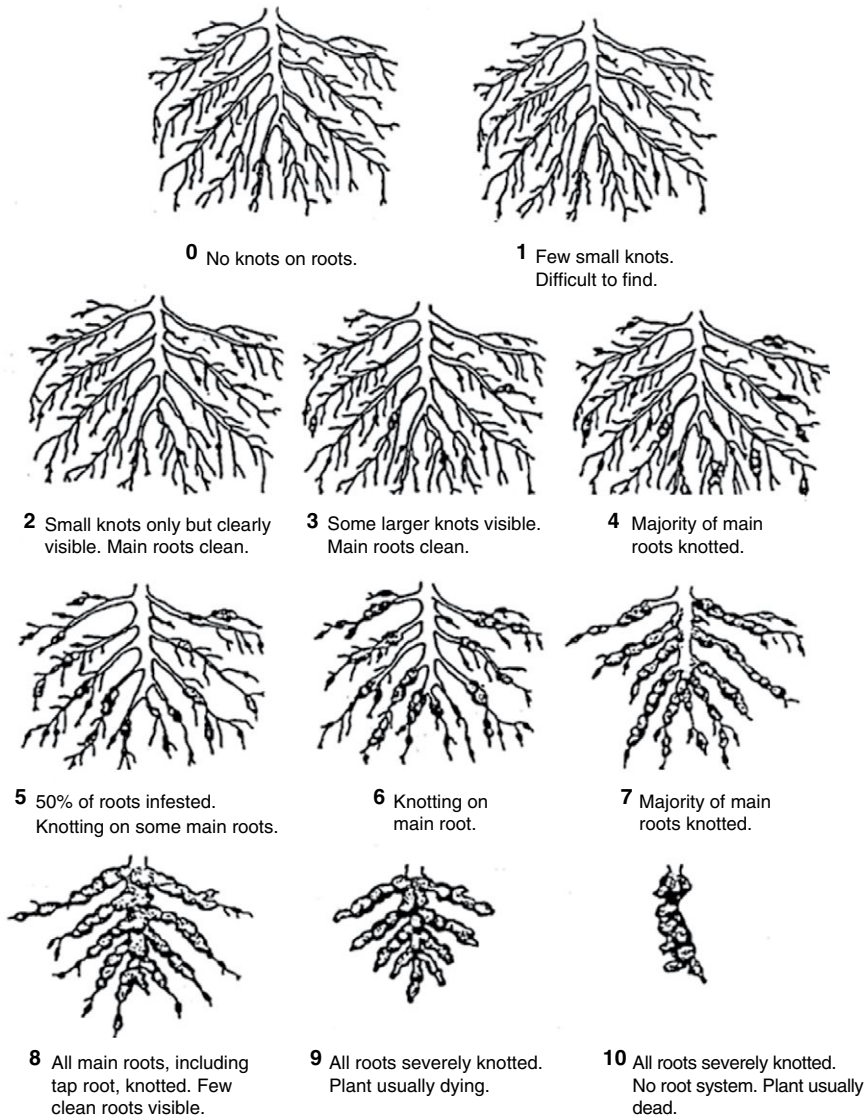


Fig. 5.14. Root knot rating chart to determine level of damage caused to rice roots by *Meloidogyne graminicola*. (Image courtesy of J. Bridge.)

cavities and channels throughout the cortex, which become necrotic for some distance into the root (Van der Vecht and Bergman, 1952; Mathur and Prasad, 1972b; Lee and Park, 1975; Babatola and Bridge, 1980; Hollis and Keoboonrueng, 1984).

Eggs of *H. oryzae* are deposited in the roots a few days after invasion, and hatching occurs 4–6 days after deposition (Van der Vecht and Bergman, 1952; Mathur and Prasad, 1972a).

The life cycle is of variable length. In north India, it is suggested that there is only one generation of *H. oryzae* a year (Mathur and Prasad, 1972a); in Japan, two generations (Kuwahara and Iyatomi, 1970; Ou, 1985); and in Senegal, three generations (Fortuner and Merny, 1979). Maximum root populations occur between tillering and heading of the rice crop (Kuwahara and Iyatomi, 1970; Fortuner and Merny, 1979).



Fig 5.15. Yellow patch of plants infested with *Hirschmanniella oryzae* in swamp rice in Gambia. (Photograph courtesy of J. Bridge.)

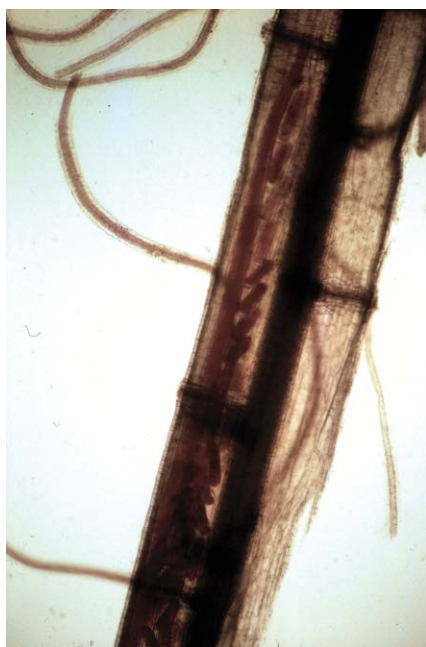


Fig. 5.16. Stained *Hirschmanniella oryzae* female and eggs in typical endoparasitic habitat in rice root. (Photograph courtesy of J. Bridge.)

Survival and means of dissemination

H. oryzae survives between crops in weeds and other hosts (Table 5.3), in ratooning rice roots and in undecayed roots of rice stubble (Mathur and Prasad, 1973b; Feng, 1986; Ichinohe, 1988). *Hirschmanniella* spp. can also survive in soil. They survive longer in roots than in soil, but survival of root populations is shorter in flooded soil due to the more rapid decay of roots. Populations of *H. oryzae* decrease slowly in wet rice fields in the absence of a host, surviving for at least 7 months (Park *et al.*, 1970), and are eradicated after 12 months (Fortuner and Merny, 1979). In dry conditions, survival is enhanced by quiescence (Fortuner and Merny, 1979); for example, *H. oryzae* can survive for longer than 12 months in soils that are not continually wet (Muthukrishnan *et al.*, 1977). *H. oryzae*, *H. imamuri* and *H. spinicaudata* have also been shown to survive in anaerobic conditions over a wide range of pH (Babatola, 1981). In fallow field soil, populations of *H. oryzae* can survive high temperatures of 35–45°C and low temperatures of 8–12°C (Mathur and Prasad, 1973b).

Hirschmanniella is spread in irrigation and flood water, and in soil adhering to implements

Table 5.3. Hosts of *Hirschmanniella* spp. parasitic on rice. (From Van der Vecht and Bergman, 1952; Kawashima, 1963; Yamsonrat, 1967; Mathur and Prasad, 1973b; Babatola, 1979; Mohandas *et al.*, 1979; Venkitesan *et al.*, 1979; Razjivin *et al.*, 1981; Edward *et al.*, 1985; Khuong, 1987; Kumar, 1990; Gao *et al.*, 1998a.)

Weeds	<i>Fimbristylis miliacea</i>
<i>Ageratum conyzoides</i>	<i>Hydrolea zeylanica</i> ^a
<i>Alternanthera sessilis</i>	<i>Ischaemum rugosum</i>
<i>Alternanthera philoxenoides</i>	<i>Ixeris denticulata</i>
<i>Astragalus sinicus</i>	<i>Leonurus artemisia</i>
<i>Bidens bipinnata</i>	<i>Lindernia antipoda</i>
<i>Boerhavia diffusa</i> ^a	<i>Ludwigia perennis</i>
<i>Brachiaria ramosa</i> ^a	<i>Mnesithea laevis</i>
<i>Coryza canadensis</i>	<i>Monochoria hastata</i> ^a
<i>Cyperus difformis</i> ^a	<i>Monochoria vaginalis</i>
<i>Cyperus elatus</i>	<i>Nelumbo nucifera</i>
<i>Cyperus nutans</i>	<i>Polygonum plebeium</i> ^a
<i>Cyperus iria</i>	<i>Polygonum hydropiper</i>
<i>Cyperus procerus</i>	<i>Scirpus articulatus</i>
<i>Cyperus pulcherrimus</i>	<i>Sesbania aculeata</i> ^a
<i>Cyperus rotundus</i> ^a	<i>Sporobolus indicus</i>
<i>Digitaria sanguinalis</i>	<i>Vallisneria spiralis</i>
<i>Echinochloa colona</i> ^a	Crops
<i>Echinochloa crus-galli</i> ^a	<i>Oryza sativa</i> ^a
<i>Eclipta alba</i> ^a	<i>Abelmoschus esculentus</i>
<i>Eclipta prostrata</i>	<i>Gossypium hirsutum</i>
<i>Eichhornia crassipes</i>	<i>Hordeum vulgare</i>
<i>Eleocharis spiralis</i> ^a	<i>Lycopersicon esculentum</i>
<i>Eleusine indica</i> ^a	<i>Pennisetum typhoides</i>
<i>Eragrostis pilosa</i>	<i>Saccharum officinarum</i>
<i>Fimbristylis ferruginea</i> ^a	<i>Triticum aestivum</i>
<i>Fimbristylis globulosa</i>	<i>Zea mays</i>

Note: ^aPlants supporting high nematode populations.

and field workers. Where there is a long history of rice cultivation, the nematodes are likely to be widespread. In Japan, for example, virtually every rice paddy is infested with either *H. imamurai* or *H. oryzae* (Ichinohe, 1988). The nematodes are also disseminated to the field in the roots of rice seedlings from nurseries. *Hirschmanniella* spp. are unusual nematodes, being perfectly adapted to constant flooding (Fortuner and Merny, 1979).

Alternative hosts

Hirschmanniella spp. are parasites of a considerable number of rice field weeds (Van der Vecht and Bergman, 1952), mainly of the families Cyperaceae and Gramineae (Table 5.3). Few cultivated crops are hosts of *H. oryzae* in India (Mathur and Prasad, 1973b); however, some crop plants are hosts of *Hirschmanniella* spp. (Babatola, 1979).

Disease complexes

Necrotic areas develop around nematodes as they migrate and feed on cortical tissues, but diminish as nematodes penetrate deeper into the roots. This suggests a phoretic relationship between the rice root nematodes and soil microorganisms, as necrosis does not occur at all in the absence of these organisms (Babatola and Bridge, 1980). Similarly, 'root browning' of rice, caused mainly by soil microorganisms, is increased in the presence of *H. oryzae* (Lee and Park, 1975).

Economic importance

It has been estimated that *Hirschmanniella* spp. infest 58% of the world's rice fields, causing 25% yield losses (Hollis and Keoboonrueng, 1984).

However, there are discrepancies in yield loss estimates around the world and suggestions that yield reductions occurring in the presence of *Hirschmanniella* are not always solely attributable to the nematodes. In Japan, for example, it has not always been possible to demonstrate high correlations between nematode population levels and yield reductions (Ichinohe, 1988). Similarly in the Côte d'Ivoire, where nematicide treatments against *H. spinicaudata* increased rice yields by 20–53%, there was no significant correlation between yields and nematode populations. This could be due to the fact that the nematicide used was only nematostatic and did not cause mortality. Another explanation is that there is a bacteriological factor present that suppresses both nematodes and rice yields (Cadet and Quénehervé, 1982). Contrasting evidence in Senegal in microplots has established that *H. oryzae* can cause a yield loss of 42% when fertilizers are not applied, with nematode populations at harvest of 3200–6000 nematodes/100 ml soil and 5–30 nematodes/g of root. Even when rice is grown in the best conditions with adequate fertilizers, yield losses are 23%, with nematode populations at harvest of 1500–2500/100 ml of soil and 90–410 nematodes/g of root (Fortuner, 1974, 1977, 1985).

Experiments with *Hirschmanniella* spp. have established varying degrees of yield loss. Inoculations of one and ten *H. oryzae*/g of soil caused 27 and 39.4% yield loss, respectively (Jonathan and Velayutham, 1987), and the numbers of panicles and grain weight were reduced by 16 and 32%, respectively, with a population level of 1200 *Hirschmanniella*/plant (Yamsonrat, 1967). *H. imamuri*, *H. oryzae* and *H. spinicaudata* reduced yields by 31–34.3% at population levels of 1000 nematodes/plant or 500 nematodes/100 ml soil (Babatola and Bridge, 1979). The yield of plants inoculated with 5000 *H. mucronata*/plant at 1 and 40 days was reduced by 50.6 and 45.6%, respectively (Panda and Rao, 1971). *H. oryzae* populations of 100/plant reduced grain yield by 35% (Mathur and Prasad, 1972b). In microplots, natural populations of 29–68 *H. oryzae*/500 ml of soil at transplanting reduced grain weight by 13.8–19.2% (Venkitesan *et al.*, 1979).

In Vietnam, economic damage by *Hirschmanniella* spp. occurs when 40 or more nematodes are present in a rice hill 1 week after transplanting; equivalent after multiplication to 800 nematodes

per hill at heading (Khuong, 1987). Yield losses caused by *Hirschmanniella* spp. are influenced by soil fertility (Fortuner and Merny, 1979), the age of the plant when infected (Panda and Rao, 1971), the number of crops and flooding (Khuong, 1987), and seasonal climatic conditions (Mathur and Prasad, 1972b).

Management measures

Management of *Hirschmanniella* spp. has been achieved by various practices, in particular fallow, weed control, use of resistant cultivars, rotation with non-host plants, chemical soil treatment of nurseries and fields, and chemical root dipping and seed coating.

CULTURAL PRACTICES: yield losses due to *Hirschmanniella* spp. are greater in poor soils. It is, therefore, possible to reduce yield losses by improving the nutritional status of the soil (Mathur and Prasad, 1972b). Nematode populations decline in the absence of host plants, but a considerable percentage can survive, depending on environmental conditions (Van der Vecht and Bergman, 1952; Mathur and Prasad, 1973a; Muthukrishnan *et al.*, 1977). Prolonged fallows might control *Hirschmanniella*, but the evidence suggests that fallows would need to be at least 12 months in wet conditions and longer in dry. They would also need to be free of other crop and weed hosts. The management of weeds, which are generally good hosts, will reduce nematode populations both in the absence of rice and during growth of the crop. Time of transplanting can be important and, in the Punjab, India, there is less build-up of *H. oryzae* when basmati rice is transplanted later in mid-July compared with mid-June (Randhawa *et al.*, 1991).

Rotation of crops is not possible in continuous rice cropping, but is often normal practice where a single wet-season rice crop is followed by dry-season crops. In fields with a single rice crop, populations of *Hirschmanniella* are always low in some localities (Khuong, 1987). This is due to a combination of dry soil and non-host, dry-season crops such as cowpea, pigeonpea, soybean, groundnut, sweet potato, sorghum, finger millet, tobacco, cabbage and onion against *H. oryzae*, *H. imamuri* and *H. spinicaudata* (Mathur and Prasad, 1973b; Babatola, 1979; Gao *et al.*, 1998a,b), and millet, cotton and wheat against

H. oryzae in India (Mathur and Prasad, 1973b). Any of these or other non-host crops in rotation with rice should reduce the risk of *Hirschmanniella* damage, but their host status may vary with different nematode species.

Three green manure legume crops, *Sesbania rostrata*, *Sphenoclea zeylanica* and *Aeschynomene afraaspera*, can give good, practical control with the additional benefit of increased soil nitrogen (Mohandas *et al.*, 1981; Germani *et al.*, 1983; Hendro *et al.*, 1992; Prot, 1992). The yield of rice following *Sesbania* was increased by 214% in microplots compared with repeated rice cropping. *Sphenoclea* can give 99% control of *Hirschmanniella* spp. *Sesbania* appears to act as a trap crop (Germani *et al.*, 1983), while *Sphenoclea* produces toxic plant exudates (Mohandas *et al.*, 1981). Unfortunately, *S. rostrata* is a very good host of the rice root knot nematode, *M. graminicola*, and it should be used with caution for the management of rice nematodes (Prot, 1994b).

Oil cakes used as organic amendments, particularly those of castor (*Ricinus communis*) and neem, can reduce populations of *H. oryzae* significantly (Jonathan and Pandiarajan, 1991; Khan and Shaukat, 1998).

Other cultural measures to alleviate damage by *Hirschmanniella* spp. in Japan are: (i) early planting and (ii) direct sowing, which both reduce initial infection (Sato *et al.*, 1970; Nakazato *et al.*, 1964 quoted in Fortuner and Merny, 1979).

RESISTANCE: the majority of rice cultivars tested are good hosts of *Hirschmanniella* spp. These include cultivars from India, Korea, Japan, Nigeria, El Salvador, Iraq, Ecuador, Thailand and Vietnam. In Korea, all 270 cultivars tested were susceptible to *H. oryzae*, although six supported only low numbers (Park *et al.*, 1970). Cultivars supporting relatively low nematode numbers have been rated as resistant (Arayaungsarit *et al.*, 1986; Rao *et al.*, 1986a). Some of these could be truly resistant, such as cv. TKM9 to *H. oryzae* from India (Ramakrishnan *et al.*, 1984). Because of the widespread occurrence of *Hirschmanniella* in rice fields, for example from all locations in Thailand (Yamsonrat, 1967) and virtually every rice paddy in Japan, it is possible that the rice cultivars that now grow best in paddies are those that are relatively resistant to, or tolerant of, *Hirschmanniella* spp. (Ichinohe, 1988).

CHEMICAL: high yield increases have been achieved using chemicals against *Hirschmanniella*, but there is little indication that chemical control is economical or practical except in special circumstances (Ichinohe, 1972).

Most of the available chemicals with nematocidal action have been applied with varying success against *Hirschmanniella*, especially in India (Edward *et al.*, 1985; Rao *et al.*, 1986a), and also in Japan (Ichinohe, 1988), Thailand (Taylor, 1969) and Côte d'Ivoire (Cadet and Quénéhervé, 1982). Chemical control has been attempted by application to field and nursery soil, as root dips and for soaking seeds. In field soil, various methods of application have been tried, including soil incorporation, application in standing water and 'mud ball' application (Prasad *et al.*, 1986a). Bare root dips for transplanted seedlings in a range of nematicides can reduce *H. oryzae* populations and increase yields (Lahan *et al.*, 1999). The mode of action of nematicides determines whether only penetration and development over a short amount of time occurs or the nematodes are actually killed and overall populations reduced (see Chapter 23, this volume).

***Heterodera* species**

The four main cyst nematodes infecting rice are *Heterodera oryzicola*, *Heterodera elachista*, *Heterodera oryzae* and *Heterodera sacchari*. The cyst nematode *H. oryzicola* is found only on upland rice widely distributed in India (Rao and Jayaprakash, 1978). *H. elachista* was first reported on upland rice in Japan (Okada, 1955), and since then reported on rice from China (Ding *et al.*, 2012; Zuo *et al.*, 2014). *H. oryzae* occurs on lowland rice in parts of the Côte d'Ivoire, Senegal (Fortuner and Merny, 1979), in Bangladesh (Page and Bridge, 1978), Nepal (Sharma *et al.*, 2001) and Iran (Pedramfar *et al.*, 2001). *H. sacchari* occurs on upland and flooded rice throughout West Africa (Côte d'Ivoire, Ghana, Guinea, Benin, Togo, Nigeria and Liberia) (Babatola, 1984; Lamberti *et al.*, 1991; Coyne *et al.*, 1996, 1999; Coyne and Plowright, 2000). Additional species of minor importance include *Heterodera mothi* and *Heterodera graminis*, found in Nepal in fields cropped with rice and wheat (Sharma *et al.*, 2001), and *Heterodera skohensis*, reported from rice fields in India (Kaushal *et al.*, 2000).

Furthermore, rice is reported to be a good host for *Heterodera sorghi* (Srivastava and Sethi, 1987).

Symptoms

The symptoms of infection by each species are similar. Root growth is suppressed, and infected roots turn brown or black. Lemon-shaped, white females and brown cysts can be observed protruding from infected roots (Fig. 5.17). Rice responds to *H. sacchari* by the proliferation of secondary roots, which have a compensatory function (Babatola, 1983a), but generally the reduced size and function of cyst nematode infected roots leads to leaf chlorosis and slowed plant growth and development, i.e. stunting and reduced tillering. Seedlings are usually more vulnerable, and Jayaprakash and Rao (1984) have observed seedling death in patches heavily infested by *H. oryzae*.

Biology

H. oryzicola and *H. elachista* are parasites of upland rice, and *H. sacchari* is damaging only in upland rice (Babatola, 1983a), although it is also found in flooded conditions. *H. oryzae* differs by its adaptation to flooding, and second-stage juveniles of *H. oryzae* can survive better in anaerobic than in aerobic water (Reversat, 1975).

The biology is as described in Chapter 2 of this volume. Females of *H. oryzicola*, *H. elachista* and *H. oryzae* deposit many eggs into a large egg sac attached to the vulval cone. Juveniles in egg sacs hatch freely in water, but there is evidence that exudates from actively growing roots are required to stimulate hatch from cysts of *H. oryzicola*

(Jayaprakash and Rao, 1982b) and *H. oryzae* (Merny, 1966). These differences in hatching behaviour indicate that J2s from later-generation egg sacs invade rice during crop growth, and that cysts are principally a means of survival (Fig. 5.18). In contrast, *H. sacchari* rarely has an egg sac and eggs hatch freely in water. *H. sacchari* also differs from the other rice cyst nematodes as it is a parthenogenetic triploid, the others being amphimictic. The life cycle of each species is complete in 24–30 days, which allows multiple generations, depending on the duration of the crop; *H. oryzicola* is said to have 12 generations/year in continuous rice, while *H. oryzae*, *H. elachista* and *H. sacchari* have 2–3 generations per crop (Berdon and Merny, 1964; Merny, 1966, 1972; Netscher, 1969; Netscher *et al.*, 1969; Nishizawa *et al.*, 1972; Shimizu, 1977; Jayaprakash and Rao, 1982a; Sharma and Swarup, 1984). The life cycle of *H. elachista* is complete in 22 days at 30°C in China (Ding *et al.*, 2012). *H. oryzicola* is dependent on rice root diffusates to induce substantial egg hatch; this is not the case with *H. sacchari*, which will hatch in water (Ibrahim *et al.*, 1993).

Alternative hosts

H. oryzicola and *H. oryzae* have a narrow host range, with many wild and cultivated Gramineae being non-hosts (Merny and Cadet, 1978; Sharma and Swarup, 1984). *H. oryzicola* has some weed hosts, e.g. *Cynodon dactylon* and *Brachiara* sp. (Charles and Venkitesan, 1985); and some Cyperaceae, e.g. *Mariscus umbellatus* and *Kyllinga monocephala* (syn. *Rhynchospora colorata*), are hosts of *H. oryzae* and *H. oryzicola* (Merny



Fig. 5.17. *Heterodera oryzicola* brown cyst (right) and young white female (left) on rice roots. (Photograph courtesy of J. Bridge.)

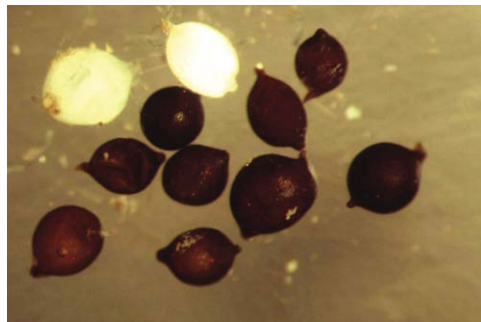


Fig. 5.18. *Heterodera sacchari* lemon-shaped cysts and white females. (Photograph courtesy of J. Bridge.)

and Cadet, 1978; Charles and Venkitesan, 1990). Surprisingly, banana is a good host of both nematodes (Taylor, 1978; Charles and Venkitesan, 1985, 1990). In this respect, *H. sacchari* is again quite distinct as it has a wide host range, including many wild Cyperaceae and Gramineae indigenous to West African savannah and humid lowlands (Odihirin, 1975).

Economic importance

Because of their restricted distribution, cyst nematodes on rice are largely of local importance. Shimizu (1977) notes that damage by *H. elachista* varies between years, and this is likely to be true for the other species, as local climatic and edaphic factors, and cultural practices, vary (Coyne *et al.*, 1998). Shimizu (1971) considered that *H. elachista* was important in later growth (presumably grain filling and maturation) and could decrease yield by 7–19%. In China, higher yield losses were estimated at 19.3–24.2%, caused by *H. elachista* (Ding Zhong, personal communication). In India, higher yield losses (17–42%) are attributed to *H. oryzae* (Kumari and Kuriyan, 1981). *H. oryzae* is a minor problem in Senegal and Côte d'Ivoire, and is replaced by *H. sacchari* in mixed populations; its importance on rice crops in Bangladesh requires assessment. *H. sacchari* populations in Côte d'Ivoire increased rapidly with intensive wet-season rice cropping, leading to yield losses of 50% (Coyne and Plowright, 1998). Coyne and Plowright (2000) demonstrated that such losses in *O. sativa* were correlated with barely detectable pre-sowing nematode population densities.

Molecular method of diagnosis

A rapid molecular diagnosis method for *H. elachista* was developed with species-specific primers with a specific fragment length of 281 bp. This method detects one out of 256 nematode specimens and one individual J2 mixed in 0.1 g rice root tissues (Wang *et al.*, 2014a,b). Combining those species-specific primers with universal primer pairs D2A and D3B was developed to detect *H. elachista* directly from total samples primarily separated from field soil (Wang *et al.*, 2014a,b). The phylogenetic tree based on ITS-rDNA shows that the Chinese populations of *H. elachista* are in the same monophyletic clade as populations from other countries (Zuo *et al.*, 2014).

Management measures

CULTURAL PRACTICES: exploiting the narrow host range of *H. oryzae*, *H. elachista* and *H. oryzae* through rotation with non-host crops is likely to be beneficial; for example, rotation with soybean or sweet potato to control *H. elachista* has given yield improvements of 2.8- to 3.7-fold (Nishizawa *et al.*, 1972). However, the traditional long fallow periods in forest and forest savannah of the Côte d'Ivoire did not clearly influence the prevalence of *Heterodera* (Coyne *et al.*, 1998). Experimentally, Coyne and Plowright (1998) managed *H. sacchari* using solarization.

RESISTANCE: rice cultivars vary in their susceptibility to *H. oryzae* (Merny and Cadet, 1978), *H. sacchari* (Babatola, 1983b) and *H. oryzae* (Jayaprakash and Rao, 1983), but few have complete resistance. The African rice *O. glaberrima* is resistant to *H. sacchari* (Coyne *et al.*, 1999). The resistance is qualitative and inherited in progeny from interspecific crosses with *O. sativa* (Plowright *et al.*, 1999). Ashurst *et al.* (2001) showed that resistance was controlled by a single recessive gene identified by Lorieux *et al.* (2003) as Hsa-10g. Microsatellite markers have been found linked to resistance and the resistance gene (Lorieux *et al.*, 2003).

Cultivars of *O. sativa* rarely have multiple nematode resistance; cvs. LaInakanda, CR143-2-2 and TKM6, although resistant to *H. oryzae*, are susceptible to *M. graminicola* (Prasad *et al.*, 1986c). *O. glaberrima*, on the other hand, is resistant to both *H. sacchari* and *Meloidogyne* spp., but resistance is under different genetic control and is not inherited equally by the progeny of interspecific crosses with *O. sativa* (Plowright *et al.*, 1999).

Pratylenchus species

Ten species of root lesion nematodes have been reported on rice throughout the world. The most common are *Pratylenchus zeae*, found in Africa, North, Central and South America, Australia, South and South-east Asia, and *Pratylenchus brachyurus*, reported from Africa, South America, Pakistan and the Philippines. They occur predominantly on upland rice, and only *P. zeae* and *Pratylenchus indicus*, a species found in India and Pakistan, have been reported to cause damage.

Symptoms

There are generally no specific above-ground symptoms of infection by *P. zae* (Plowright *et al.*, 1990). However, the leaves of 22-day-old rice seedlings infected with *P. indicus* are said to yellow from the tip, wilt and dry (Rao and Prasad, 1977). *Pratylenchus* spp. cause discrete lesions in the root cortex that become necrotic and coalesce as infection spreads. Root size and function are diminished, either tillering or shoot extension is reduced and plants become stunted. *Pratylenchus* is said to be associated with a disease known as entorchamiento in Colombia; the symptoms are stunted growth, twisting and yellowing of leaves and proliferation of deformed secondary roots (Pardo and Munoz, 1994).

Biology

Population levels of *P. indicus* decline rapidly during the fallow periods and persist in low numbers (Prasad and Rao, 1978a). *P. zae* can survive in a cultivated clean fallow for up to 6 months. Weed hosts of *P. zae* are *C. dactylon*, *Amaranthus spinosus*, *Dactyloctenium aegyptium*, *Digitaria sanguinalis* and *Echinochloa* sp. (Fortuner, 1976).

Invasion by *P. zae* takes place within 1 week of emergence, the life cycle being completed in about 30 days. *P. indicus* completes a life cycle in 33–34 days, and several overlapping generations occur on a single crop (Prasad and Rao, 1982a). The optimum temperature for *P. indicus* reproduction is 23–30°C, and population peaks are always preceded immediately by rainfall (Prasad and Rao, 1979a). During crop growth, *P. zae* is found mainly in rice roots, and soil population levels are generally low. Plowright *et al.* (1990) found that the rate of *P. zae* reproduction was greatest after flowering, and numbers increased towards grain maturity. *P. zae* migrates into the soil from heavily infected necrotic roots. *Pratylenchus* spp. are readily disseminated in soil and infected root material.

Economic importance

Despite the prevalence of *P. zae* in upland rice, there is very little information on its pest status. However, in South-east Asian upland rice eco-systems, *Pratylenchus* spp., together with *Meloidogyne* spp., are potentially the most economically

important nematode pests (Prot *et al.*, 1996). Plowright *et al.* (1990) have shown that rice yield can be increased 13–29% by control of *P. zae*, but some cultivars may be tolerant of infection. The maximum yield reduction in the field was 30% with an infection of 1000 *P. zae*/g of root at harvest, and higher nematode densities at harvest will not necessarily cause further yield loss. Martin (1972) reported that the growth of rice infected with more than 500 *Pratylenchus* sp. (probably *P. zae*)/g of root was poor, and severely stunted plants had more than 3500 nematodes/g of root. Prasad and Rao (1978b) found that the yield of rice cv. Bala was reduced by 33% at final population densities of *P. indicus* up to 1625/g of root. The data suggest that *P. zae* and *P. indicus* can cause yield loss in upland rice, but further studies are required.

Management measures

P. zae can be managed effectively using nematocides (Plowright *et al.*, 1990; Sahoo and Sahu, 1993a). However, chemical control in upland rice requires economic appraisal. Control through crop rotation has been reported using poor or non-host crops such as mung bean, black gram, cowpea and sesame (Prasad and Rao, 1978a). The yield of rice after rice in fields heavily infested with *P. zae* was 37% lower than the yield of rice after cowpea, but two successive croppings with resistant legume crops are necessary to reduce nematode populations to a low level, and this rotation will protect only one rice crop from the nematode (Aung and Prot, 1990). However, *P. zae* has a wide host range, and many of the food crops (mainly cereals) grown in upland rice cropping systems are good hosts (Table 5.4), as also are the many weeds and other types of wild rice found in upland rice fields (Sahoo and Sahu, 1993b). Fallow periods of a practical length will reduce, but not eliminate, damage by *P. zae* to susceptible intolerant cultivars.

Differences in susceptibility of rice cultivars and accessions to *P. zae* (R.A. Plowright and D. Matias, unpublished data) and *P. indicus* (Prasad and Rao, 1982b) have been found, but no useful resistance has yet been identified. Upland rice cultivars appear to differ in their tolerance of *P. zae* (Plowright *et al.*, 1990); if this is a reliable and heritable trait, then it will be useful for alleviating yield loss.

Table 5.4. Some important hosts of *Pratylenchus* *zeae*.

<i>Oryza sativa</i>	<i>Vigna unguiculata</i>
<i>Oryza glaberrima</i>	<i>Lycopersicon esculentum</i>
<i>Oryza breviligulata</i>	<i>Ipomoea batatas</i>
<i>Eleusine coracana</i>	<i>Glycine max</i>
<i>Sorghum bicolor</i>	<i>Arachis hypogaea</i>
<i>Zea mays</i>	<i>Saccharum</i> spp.
<i>Triticum aestivum</i>	<i>Solanum tuberosum</i>
<i>Avena sativa</i>	<i>Allium cepa</i>
<i>Hordeum vulgare</i>	<i>Lactuca sativa</i>
<i>Secale cereale</i>	<i>Nicotiana tabacum</i>
<i>Amaranthus</i> sp.	<i>Gossypium</i> spp.

Criconemoides* (*Criconemella*) and *Criconema

Criconemoides (= *Criconemella*) spp. (*Criconemoides annulatus*, *Criconemoides curvatus*, *Criconemoides incisus*, *Criconemoides informis*, *Criconemoides obtusicaudatus*, *Criconemoides onoensis*, *Criconemoides ornatus*, *Criconemoides oryzae*, *Criconemoides palustris*, *Criconemoides paragoodeyi*, *Criconemoides rusticus*, *Criconemoides sphaerocephala* and *Criconemoides tescorum*) and *Criconema crassianulatum*, *Criconema corbetti*, *Criconema jaejuense* and *Criconema cardamomi* occur on upland and flooded rice in various areas of the world (Bridge *et al.*, 2005). In Louisiana, *C. onoensis* decreased rice production by 15%, and *C. onoensis* populations as high as 420/100 ml of soil reduced yields of upland rice in Mauritius (Chinappen *et al.*, 1988).

Hoplolaimus

A number of *Hoplolaimus* spp. are found on upland rice; *Hoplolaimus indicus*, a migratory endoparasite, is reported to be a damaging parasite of rice in India and Nepal (Das and Rao, 1970; Sharma *et al.*, 2001), and another species, *Hoplolaimus clarissimus*, is associated with damage to rice in Togo, where rice is cropped continuously on the same fields (Coyne *et al.*, 1996).

Damage by *H. indicus* is not always obvious in the field and, in the early seedling stage, is very similar to nitrogen deficiency. Leaves of seedlings infected by *H. indicus* are yellowish before turning brown and brittle, with ash-coloured tips. Plants

are stunted, with shortened upper internodes; new leaves can be curled. The symptoms can be less apparent in the latter stage of the crop (Banerji and Banerji, 1966; Das and Rao, 1970). Rice roots have brown lesions at invasion points. Cavities can be found in the cortex, cells lose their rigidity, vascular elements become distorted and roots become flaccid (Das and Rao, 1970; Ramana and Rao, 1975; Alam *et al.*, 1978).

There are few studies of the yield losses caused by *H. indicus* in the field, but, in pot experiments, initial population levels of 100–10,000 nematodes/plant can reduce numbers of tillers by 21.5–36.0% and reduce grain yields by 10.7–19.8% (Ramana and Rao, 1978).

Paralongidorus*, *Longidorus

Four species of *Paralongidorus* have been recorded on flooded rice: *Paralongidorus oryzae* occurs in Nepal and India (Verma, 1973); *Paralongidorus lutensis* and *Paralongidorus zenobiae* are found on deepwater rice in Bangladesh (Hunt and Rahman, 1991); *Paralongidorus australis* is a recognized, locally important parasite of rice in north Queensland, Australia (Stirling and Vawdrey, 1985).

In the field, *P. australis* causes poor growth, mainly in rice planted during the summer. The first symptoms appear 7–10 days after flooding and develop into patches of stunted yellow plants, of which many may die. Primary roots show brown necrotic tips, sometimes hooked or curled; secondary roots are shorter than normal, often with a forked appearance. The root system is severely reduced, attacked roots being 1–5 cm long versus 15–20 cm in healthy plants (Stirling and Shannon, 1986). Experimentally inoculating rice seedlings with 250–900 nematodes/plant produces symptoms of damage (Stirling, 1984).

Control can be achieved by increasing the rate of nitrogenous fertilizer in combination with deep ploughing (>40 cm), or by changing to moist cultivation rather than flooded in order to inhibit nematode movement (Stirling and Shannon, 1986). Delaying flooding after sowing decreases the degree of nematode damage (Stirling *et al.*, 1989). Control by dry fallow is effective but not normally appropriate because *P. australis*

can remain anabiotic for several years. Crop rotation with maize, sorghum or soybeans may be a preferable substitute for fallow. No resistance has been found, but some rice cultivars are more tolerant than others (Stirling *et al.*, 1989).

Other Nematodes

Many nematodes, in addition to those already discussed, are found with rice (Fortuner and Merny, 1979), but few of these are reported to be associated with damage, and are probably of minor or local importance.

Conclusions and Future Prospects

Most rice nematodes are potentially damaging, but their economic importance is influenced strongly by the environment. With some widespread nematodes, such as *A. besseyi*, the damage they cause is not proportional to their distribution; for others, such as *Hirschmanniella* spp., yield losses are probably underestimated. The damage caused by *D. angustus* can be devastating, but it has a limited distribution and its occurrence is unpredictable. Furthermore, as new rice cultivars are bred and regional cropping practices change, nematodes may emerge to be even more important. An ominous example of this is the spread of *D. angustus* from its traditional host, deepwater rice, to the more widely grown and globally important irrigated and lowland rice. Other new nematode problems are surfacing; for example, *Paralongidorus*, at present only known to be damaging in Australia, and *Aorolaimus* in Africa. *Paralongidorus* in particular could be more widespread on rice and may have avoided detection as it is difficult to isolate.

The management of rice nematodes poses a number of problems, primarily because measures to control one nematode may increase the damage caused by another. This complicates the recommendation of cultural methods for nematode control on rice and other crops in a rice cropping system; for example, flooding reduces or eliminates populations of *Pratylenchus*, *Hoplolaimus*, *Heterodera* and most *Meloidogyne* spp., but encourages *Hirschmanniella* spp. Significant reductions in populations of *Hirschmanniella* attacking rice and in soil

populations of *Meloidogyne* spp. damaging vegetables can be achieved where irrigated or lowland rice is rotated with upland vegetable crops. However, this same system would increase damage to and yield loss of rice by *M. graminicola*. An accurate knowledge of the species present in a field is thus an important prerequisite for investigating such control methods. Chemical control of rice nematodes will rarely be economic, and the dangers of exposure to and difficulties of applying nematicides in flooded rice are self-evident. In flooded soils, sulfur dioxide, produced by anaerobic bacteria, could be used as a form of nematode control, and some trials have proven the efficacy of such phenomena (Jacq and Fortuner, 1979). The difficulty is that rice seedlings may also be killed. More research on this and other similar techniques could be beneficial, but requires the cooperation of nematologists, agronomists and soil microbiologists. Harvesting solar energy through soil solarization of nursery beds prior to sowing seed, summer ploughing of the main field, incorporation of organic manures and crop residues and suitable crop rotation can help in developing location-specific integrated nematode management methods. Some useful molecular studies have been carried out to identify resistance genes and understand the mechanism of tolerance to abiotic and biotic stresses including insects, fungi, bacteria and nematodes. Cultivars with resistance or tolerance to nematodes could offer acceptable and economic management of rice nematodes, but there are few, if any, ongoing rice nematode resistance breeding programmes. There is some information on the variation in the susceptibility of rice cultivars to most rice nematodes, but essentially very little is known about the mechanisms and inheritance of resistance. Progress is being made with some of the important rice nematodes, but a coordinated international effort is required by nematologists, agronomists and plant breeders to identify and transfer resistance to commercially acceptable rice cultivars.

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6 Nematode Parasites of Cereals*

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Cereals constitute the world's most important source of food and fodder, with wheat, maize and rice occupying the top positions in terms of production, acreage and sources of nutrition (Table 6.1), particularly in developing countries. During 2010–2014, more than 75% of the land cultivated for food crops was devoted to cereal crops (<http://www.fao.org/faostat/en/#data/QC>, accessed 10 January 2018).

The improved yield of new cultivars has boosted agricultural output, but yield potential is often far below theoretical maximum yields, mainly due to production constraints. Insect pests and diseases are important constraints of crop production, though extensive research on plant parasitic nematodes is lacking. Most nematodes are microscopic and remain subterranean, and consequently are difficult to recognize and control.

Approximately 10% of global crop production is lost to nematode damage (Whitehead, 1998), at a cost of approximately US\$80 billion per annum (Nicol *et al.*, 2011). This figure is likely underestimated, since little is understood about the presence and impact of plant parasitic nematodes, particularly in developing countries. For instance, a survey of 120 households in Uganda revealed that only 19% of farmers knew about nematode pests and the damage they caused to maize crops (Kagoda *et al.*, 2010).

Nematodes are usually managed with just one method (dependent on the target pest and resources available), though increasingly, multiple strategies are being implemented. Commonly practised methods include crop rotation, intercropping, use of resistant and tolerant genotypes, and cultural practices. This chapter provides an insight into the economically important nematode pests of cereal grains other than rice (*Oryza sativa*), which is covered in Chapter 5, this volume. It details the distribution, biology and life cycle, damage potential and economic importance of major nematode pests, and viable management options for their control.

For further information about the nematode pests discussed in this chapter, see useful reviews by De Waele and McDonald (2000), Kollo (2002), Nicol (2002), Bridge and Starr (2007), Riley *et al.* (2009), Dababat *et al.* (2015a,b) and McDonald *et al.* (2017).

Wheat and Barley

In 2016, an estimated 742 million tons of wheat (*Triticum aestivum*) was produced on more than 223 million ha (www.fao.org/worldfoodsituation/csdb/en/), more than half of the area allocated to crops (Breiman and Graur, 1995). In 2050,

*A revision of the chapter by A.H. Mc Donald and J.M. Nicol in the second edition.

Table 6.1. Contribution of cereals in world food production (1000 t) during the period 2010–2014. (From the FAO website.)

Continent	Maize	Rice	Wheat	Barley	Sorghum	Millet	Oat	Rye	Total cereals
World	933,985	727,079	690,645	135,339	60,559	28,041	22,055	14,316	2,612,019
Africa	70,162	28,089	25,079	6,411	25,080	12,425	250	97	167,593
Asia	284,440	657,401	307,637	19,983	9,943	14,587	1,051	1,139	1,296,181
Australia	616	763	25,957	8,418	1,857	40	1,256	36	38,943
Europe	107,979	4,211	219,493	82,660	1,014	724	13,838	12,573	442,492
North and Central America	361,513	10,677	89,917	13,002	15,624	254	4,110	417	495,514
South America	108,520	24,424	22,561	4,864	6,910	9	1,547	55	168,890

the world's population is expected to reach 9 bn; thus, it is estimated that cereal production needs to increase by 50% by 2030 (Alexandratos and Bruinsma, 2012). These production increases will occur by expanding the wheat area and improving the yield per unit area sown. The rate of production increase will slow, due to the scarcity of new land for cultivation or irrigation. However, the gap between yield potential and actual yield will reduce, and it is anticipated that by 2030 more than half of wheat production will come from developing countries (Marathe and Gomez-MacPherson, 2001; Curtis, 2002).

Barley (*Hordeum vulgare*) is closely related to wheat. It is an ancient cereal grown in nearly all cultivated areas in temperate and in many sub-tropical areas, including high-altitude and drought-prone regions. Barley is used for human consumption, animal feed, malt production for brewing, and as a coffee substitute in many Asian countries (Newman and Newman, 2008; Arendt and Zannini, 2013).

Three other important cereals are oat (*Avena sativa*), rye (*Secale cereale*) and triticale (wheat × rye), which are used predominantly for animal feed. Oat grows best in cool, moist regions, has a high water requirement and is less sensitive than wheat or barley to soil conditions (Leonard and Martin, 1965). Rye is considered one of the most resilient cereals in poor environmental conditions and is used for grain, pasture or as green manure crops (Leonard and Martin, 1965). Triticale is less well known, though it is becoming increasingly important due to its wide adaptability in poor soils and climates. Rye offers, in many cases, a high level of resistance against cereal root nematodes. This also applies to some triticale cultivars, depending on the regions of

rye chromosomes incorporated. These two crops offer great potential in rotational control of cereal nematodes, particularly where small grain cereals are the predominant crops.

Small cereal yields are impacted primarily by the availability of fertilizer and water, although also by cultivar selection, pesticide use and management practices. These latter factors determine nematode population fluctuations and the degree of economic loss, although water and nutrient stress also increase the likelihood of nematode damage.

Nematodes of Wheat and Barley

Numerous plant parasitic nematodes are associated with wheat and barley, although only a few are considered economically important: (i) cereal cyst (CCN), *Heterodera* spp.; (ii) root lesion (*Pratylenchus*); (iii) seed gall (*Anguina tritici*); (iv) root knot (*Meloidogyne*); and (v) *Ditylenchus dipsaci* nematodes.

Cereal cyst nematodes: *Heterodera*

Distribution

Heterodera is one of the oldest known genera of plant parasitic nematodes, composed of 12 closely related *Heterodera avenae* species (Subbotin *et al.*, 2010a; Subbotin, 2015). *H. avenae* was the first to be reported and is the most economically important (Rivoal and Cook, 1993, cited in McDonald and Nicol, 2005), followed by *Heterodera latipons* (Franklin, 1969), *Heterodera hordecalis*

(Andersson, 1974), *Heterodera filipjevi* (Madzhidov, 1981) and several others (Wouts *et al.*, 1995). These species have been found in tropical and subtropical environments.

H. avenae, later described as *Heterodera australis* in Australia (Subbotin *et al.*, 2002) and recently described as *Heterodera sturhani* in China (Subbotin, 2015), is the most important cereal cyst nematode. It has been detected across Europe (Belgium, France, Greece, Italy, Portugal, Spain, UK, former Yugoslavia) and the Middle East (Egypt, Iran, Pakistan, Saudi Arabia, Syria, Turkey), as well as Australia, Canada, China, India, Israel, Japan, New Zealand, Peru, South Africa, the USA and countries in North Africa and western Asia (McDonald and Nicol, 2005; Qiao *et al.*, 2016).

Another important species, *H. latipons*, is essentially Mediterranean in distribution. It was first discovered in Israel and Libya, but since found in Cyprus, Iran, Italy, Jordan, Lebanon, Morocco, Spain, Syria and Turkey. It has also been detected in temperate continental climates in the former USSR, northern Europe, Bulgaria, Czech Republic, Japan and Canada (McDonald and Nicol, 2005; Mokrini *et al.*, 2012).

H. filipjevi, formerly known as the Gotland strain of *H. avenae* (Madzhidov, 1981), also has an increasingly wide distribution. It has been detected in Belarus, Bulgaria, China, Germany, India, Iran, Italy, Kazakhstan, Norway, Poland, Russia, Spain, Sweden, Syria, Tajikistan, Turkey, the UK, the USA, and the former USSR (Grosse and Kohlmüller, 2004; Holgado *et al.*, 2004a; Madani *et al.*, 2004, cited in McDonald and Nicol, 2005; Abidou *et al.*, 2005a; Smiley *et al.*, 2008; Mitchinson *et al.*, 2009; Subbotin *et al.*, 2010b).

Other important *Heterodera* spp. include *H. hordecalis* found in Germany, Iran, Sweden and UK (Tanha-Maafi *et al.*, 2003, cited in McDonald and Nicol, 2005; Tanha-Maafi *et al.*, 2007), *Heterodera zae* in India, Iraq and Pakistan, and various others including *Heterodera mani*, *Heterodera bifenestrata* and *Heterodera pakistanensis*, as well as an unrelated CCN, *Punctodeira punctata* (McDonald and Nicol, 2005).

Biology and life cycle

CCNs complete one generation per year; the life cycle (Fig. 6.1) begins as an egg contained in a cyst, developing through four successive juvenile stages and finally mature males and females

(see also Chapter 2, this volume). First-stage juveniles (J1) moult to J2 inside the egg. The emergence of J2 from the egg and cyst is triggered by specific interactions of soil temperature and moisture, and to some extent by exudates from host roots (Smiley and Nicol, 2009). One CCN survival strategy is that not all J2 hatch at the same time. Once hatched, CCNs begin to search for a suitable host, relying primarily on gradients of chemicals released by the host roots (Perry, 1997; Subbotin *et al.*, 2010a; Turner and Subbotin, 2013).

H. avenae and *H. latipons* infect cereal hosts using a variety of mechanisms (Mor *et al.*, 1992, 2008). *H. avenae* J2 attack the root-tip region, inducing typical branching and swelling of roots, whereas *H. latipons* J2 penetrate at sites more distant from the root tip. Thus, *H. latipons* does not produce clearly visible root symptoms in the early infection period (seedling stage). Syncytia formation also differs between the two species, and growth inhibition caused by *H. avenae* is more severe than that inflicted by *H. latipons*.

Environmental factors

Many abiotic factors, such as fertility, pH, soil type and organic matter content, influence nematode population development and damage severity (Duggan, 1961). Moderate nematode densities under favourable environmental plant growth conditions may not cause as much damage as when plant growth is restricted by moisture stress or low fertility levels (Kornobis *et al.*, 1980). Increased nitrogen application is known to reduce the intensity of nematode damage to crops, though not at high nematode densities (Germershausen *et al.*, 1976). The use of zinc chloride increased J2 hatching rates of *H. filipjevi* by 30% compared to a sterilized water control (Sahin, 2010).

H. avenae has been associated with economic damage almost exclusively in light soils. Sand particles are optimal for nematode population development and have a lower water-holding capacity. Irrespective of soil type, when the intensity of cropping exceeds a certain limit, damage is imminent (Kort, 1972).

Hatching and survival

Encysted CCN eggs can survive for several years at 5°C and low relative humidity (Kyrou, 1976;

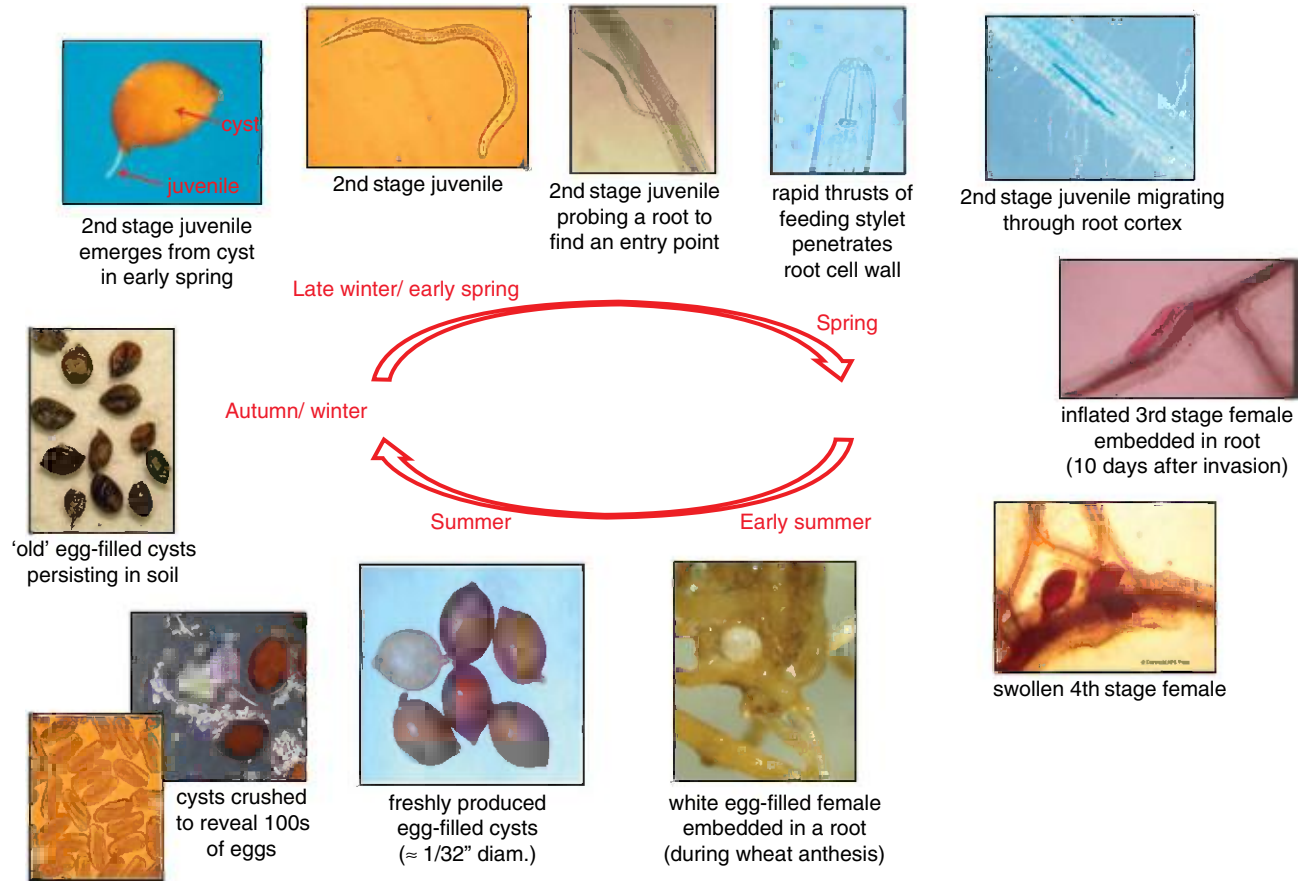


Fig. 6.1. Life cycle of the cereal cyst nematode *Heterodera avenae*. (Photograph courtesy of R. Smiley.)

Meagher, 1982; Scholz and Sikora, 2004, Sahin *et al.*, 2010). Eggs in cysts are susceptible to drying, with prolonged exposures markedly reducing J2 emergence. However, tropical populations exposed to prolonged dry summer conditions do not lose their viability. Even in the hot dry summers of Israel and India, encysted J2 remain viable until suitable temperatures for emergence occur (Minz, 1956). Depending on the CCN origin, J2 can hatch over a wide range of temperatures, though this is controlled (except for *H. filipjevi*) by diapause, even if favourable conditions return (Sahin *et al.*, 2010). *H. latipons* J2 hatch well (maximum 33%) at 10°C in Syria (Scholz and Sikora, 2004) and Jordan (Al-Abed *et al.*, 2009). Sahin *et al.* (2010) found J2 hatching of 94% for Turkish populations of *H. filipjevi* at a temperature range of 10–15°C. In Australia, *H. avenae* J2 hatch optimally at temperatures between 10 and 15°C, reaching a peak of 80% under field conditions (Banyer and Fisher, 1971; Meagher, 1977; Stanton and Eyres, 1994). Similar results were reported from France for

H. avenae (Rivoal, 1986). In Algeria, *H. avenae* J2 can hatch over a wide range of constant temperatures (3–25°C) but at different time intervals (Mokabli *et al.*, 2001).

Symptoms of damage

CCN damage is characterized by uneven patches (from 1 to 100 m² or more) of poorly growing plants, randomly distributed throughout a field (Fig. 6.2). Plant damage and the size and number of patches are directly related to nematode densities and their distribution in a field. Under monocultures, such patches coalesce and can cover the entire field uniformly within 3–4 years (Ibrahim *et al.*, 1999). Severely infected plants remain stunted and their leaves become pale yellowish-green in colour, with thin narrow leaf blades and generally fewer tillers. Ears, if formed, have very few grains.

CCN root symptoms vary depending on the host, but are recognizable in tropical areas within 1–2 months after sowing. *H. avenae*-infected



Fig. 6.2. Field symptoms of stunting and shoot chlorosis on summer wheat caused by *Heterodera avenae* infection. (Photograph courtesy of R. Smiley.)

wheat roots show increased root production (Fig. 6.3), with several females visible at each knot (Rivoal and Cook, 1993). Infected oat roots are shorter and thicker, while barley roots appear less affected. Other *Heterodera* spp. also produce host-specific symptoms on the roots of cereals. Tufting of roots may not be noticeable during field examination due to adhering soil (Holdeman and Watson, 1977).

Pathotypes

CCN populations are very heterogeneous for virulence, but also differ in their host range (Cook and Noel, 2002). Results from the International Cereal Test Assortment in 1972–1973 demonstrated the existence of more pathotypes than originally recognized, especially in subtropical regions (Cook and Rivoal, 1998; Turner and Rowe, 2006). The cv. Barley 191, which is reported to be resistant to known populations of *H. avenae* in Europe, is susceptible to *H. avenae* populations in Australia, India and Norway (Brown, 1972; Mathur *et al.*, 1974). Further, pathotypes may also occur in mixtures, which complicates description of the pathotype in a particular sample.

The pathotype scheme by Andersen and Andersen (1982) (Table 6.2) is based on known R-genes and resistance sources, but underestimates the polymorphism of resistance and avirulence genes (Cook and Noel, 2002). Cyst multiplication on resistant barley (genes *Rha1*, *Rha2* and *Rha3*) (Andersen, 1961) identified three primary pathotype groups: *Ha1* and *Ha2* are widely distributed in Europe, North Africa and Asia

(Al-Hazmi *et al.*, 2001; Cook and Noel, 2002; Mokabli *et al.*, 2002; McDonald and Nicol 2005), while *Ha3* is found mostly in Australia, Europe and North Africa (Rivoal and Cook, 1993; Mokabli *et al.*, 2002). Each pathotype group is further subdivided by their reactions on other differentials.

Mathur *et al.* (1974) previously reported five pathotypes from north India using host differentials. Swarup *et al.* (1979) and Siddiqui and Husain (1989) reported two pathotypes from India. Additionally, Andersen and Andersen (1982) concluded that three pathotypes *Ha21*, *Ha31* and *Ha41* occurred in India, while Bekal *et al.* (1998) reported an additional pathotype (*Ha71*). Also, Bishnoi and Bajaj (2000, 2002) reported pathotypes *Ha31* and *Ha41*. On the basis of detailed morphological studies, eight populations could be distinguished in two different morphological groups. One group represented *H. avenae* and the other *H. filipjevi* (Madzhidov, 1981). Stelter (1984) designated them *Hf31* and *Hf41*. Pathotype-1 (Himachal Pradesh, Ambala and Punjab populations) of *H. avenae* reported by Mathur *et al.* (1974) and Swarup *et al.* (1979) represent *H. filipjevi*, whereas other populations are *H. avenae*. Other pathotypes of *Heterodera* spp. are less well understood, although this knowledge is essential for understanding nematode biology and possible host-resistance control options.

Populations initially designated as pathotypes *Ha23* and *Ha33* (Andersen and Andersen, 1982) are now considered to be pathotypes of the closely related species *H. filipjevi* (Subbotin *et al.*, 2003; Ozarslandan *et al.*, 2010; Smiley *et al.*,



Fig. 6.3. *Heterodera avenae* damage to roots of summer wheat exhibiting typical bushy root clusters caused by nematode-induced root branching. (Photograph courtesy of R. Smiley.)

Table 6.2. Pathotypes of cereal cyst nematodes defined by an International Cereal Test Assortment of cereal cultivars. (From Cook and Rivoal, 1998.)

Pathotype	<i>Heterodera avenae</i> group		<i>Ha1</i> group		<i>Ha2</i> group		<i>Ha3</i> group		<i>Heterodera hordecalis</i>		<i>Heterodera bifenebra</i>		
	<i>Ha11</i>	<i>Ha21</i>	<i>Ha31</i>	<i>Ha41</i>	<i>Ha51</i>	<i>Ha61</i>	<i>Ha71</i>	<i>Ha12</i>	<i>Ha13</i>	<i>Ha23</i>	<i>Ha33</i>	<i>Hh1</i>	<i>Hb1</i>
Differential cereal species/ cultivars (R-gene) ^a													
Barley													
Emir (+ ex Emir)	S	S	—	S	—	R	S	S	S	S	S	S	S
Ortolan (<i>Rha1</i>)	R	R	R	R	R	R	R	S	S	S	S	S	S
Siri (<i>Rha2</i> + ex Herta)	R	R	R	S	S	S	R	R	S	S	S	S	S
Moroco (<i>Rha3</i> +)	R	R	R	R	R	R	R	R	R	R	R	R	S
Varde	S	—	—	S	—	S	S	S	S	S	S	S	S
KVL191(<i>Rha2</i> +)	R	R	R	—	S	S	S	R	—	—	—	—	—
Bajo Aragon	R	—	—	R	—	R	R	R	S	S	S	S	(R)
Herta	S	S	R	—	R	—	R	S	S	—	—	—	—
Martin 403-2 (2 dom)	R	—	—	R	—	R	R	R	R	S	S	S	S
Dalmastische	(R)	—	—	S	—	R	(S)	S	S	(R)	S	(R)	S
La Estanzuela	—	—	—	—	—	—	S	—	—	(R)	—	(R)	S
Harlan 43	R	—	—	—	—	—	R	R	—	R	S	—	—
Oat													
Sunll (minor genes)	S	R	R	R	R	S	R	S	S	S	S	R	S
Nidar	S	—	—	S	—	S	R	S	S	S	S	R	S
Pusa hybrid BS1 (1 dom)	R	R	—	R	R	R	R	R	S	R	S	R	S
Silva (>1 gene)	(R)	—	—	R	—	(R)	R	(R)	(R)	(R)	S	R	S
<i>Avena sterilis</i> 1376 (1–3 dom)	R	R	—	R	R	R	R	R	R	R	R	R	S
IGV.H 72-646	R	—	—	R	—	R	R	R	S	S	S	—	S
Wheat													
Capa	S	S	—	S	—	S	S	S	S	S	S	R	S
Loros (<i>Cre1</i> +)	R	R	—	R	—	(R)	R	R	(R)	S	S	R	(R)
Iskamish K-2-light	S	—	—	R	—	(R)	—	S	S	S	S	R	R
AUS 10894 (<i>Cre1</i> +)	R	—	—	R	—	R	S	R	(R)	S	S	R	(R)
Psathias	—	—	—	S	—	—	—	S	S	S	R	R	S

Notes: ^aResistance genes are those in italics (*Rha1*, *Rha2*, *Rha3* define the pathotype groups); dom = dominant gene; + = additional gene(s) inferred; S = susceptible; R = resistant (<5% new females on susceptible control); () = intermediate; — = no documentation. Sourced from Rivoal and Cook (1993) and Cook and Rivoal (1998), and previously modified from Andersen and Andersen (1982) and their revision.

2011; Toktay *et al.*, 2013). *H. latipons* has never been pathotyped. Due to the increasing prevalence and economic importance of the three major CCN species, more studies have investigated and identified new potential sources of resistance to control local CCN populations (Dababat *et al.*, 2016). It is thus necessary to expand and update the International Cereal Test Assortment by including local cereal genotypes (Cook and Noel, 2002; Smiley and Nicol, 2009; Smiley *et al.*, 2011; Cui *et al.*, 2015). To date, molecular technology has failed to distinguish pathotypes of CCNs and markers for virulence traits.

Identification, quantification and detection of cereal cyst nematodes

The genus *Heterodera* contains 84 species (Subbotin *et al.*, 2010b; Subbotin, 2015). Traditional identification of *Heterodera* spp. is based primarily on the morphology and morphometrics of cysts and J2 (Rivoal *et al.*, 2003). This process is time-consuming and requires specialized skills and training, especially in the case of species mixtures (Yan and Smiley, 2009; Toumi *et al.*, 2015b). Molecular techniques, such as restriction fragment length polymorphism (RFLP) of ribosomal DNA, have enabled taxonomic differentiation among several entities of the CCN complex (Bekal *et al.*, 1997; Subbotin *et al.*, 2000). However, reliable and rapid species identification is vital for the correct implementation of management strategies, monitoring and controlling the movement or introduction of potential pests.

The enzyme-staining technique for characterizing a single protein or small subset of proteins facilitates diagnosis of CCN species (Rumpenhorst, 1985; Subbotin *et al.*, 1996, 2002; Holgado *et al.*, 2004b). However, the high degrees of polymorphism within CCN populations complicate the process (Esbenshade and Triantaphyllou, 1988; Radice *et al.*, 1988; Andrés *et al.*, 2001). Moreover, the influence of sample preparation, sample storage and the developmental stages of the nematodes on the banding patterns call for more sensitive DNA-based methods.

Molecular markers (random amplified polymorphic deoxyribonucleic acid (RAPD) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of ribosomal DNA (rDNA)-internal transcribed spacer (ITS)) have enabled the identification of various CCN

species (Rivoal *et al.*, 2003; Subbotin *et al.*, 2003; Ophel-Keller *et al.*, 2008; Waeyenberge *et al.*, 2009). The analysis of coding genes (18S, 5.8S and 28S) of rDNA, and the two intermediate non-coding regions (ITS1 and ITS2), became a favoured method of nematode identification (Vrain *et al.*, 1992; Wendt *et al.*, 1993; Ferris *et al.*, 1993, 1994). Both spacers ITS1 and ITS2 are known to evolve faster and are therefore more variable than the coding genes. PCR-RFLP based on ITS regions of the rDNA repeat units of *Heterodera* spp. has frequently been used for the identification of cyst nematodes (Rivoal *et al.*, 2003; Abidou *et al.*, 2005b; Yan and Smiley, 2009). Recently, new quantitative trait loci (QTL) for CCNs have been developed by the use of association mapping techniques (Dababat *et al.*, 2016; Pariyar *et al.*, 2016a).

Recent progress in DNA sequencing allows taxonomic analysis of nematodes (Waeyenberge *et al.*, 2009). DNA sequencing costs have decreased more than 100-fold during the past decade, due to significant improvements in equipment, technology and process development for sequencing. However, nematode identification via sequencing remains relatively expensive, laborious and time-consuming (Waeyenberge *et al.*, 2009), and *in silico* studies have shown that, in some cases, identical ITS sequences are present in morphologically distinct *Heterodera* spp. such as *H. avenae* and *Heterodera arenaria* (Subbotin *et al.*, 2000).

Species-specific PCR assay is an efficient tool for accurate, rapid and sensitive detection of the three main CCN species (Yan and Smiley, 2009; Qi *et al.*, 2012; Peng *et al.*, 2013; Yan *et al.*, 2013). Two qPCR assays for *H. avenae* and *H. latipons* have also been established for quantitative detection (Toumi *et al.*, 2013a,b, 2015a).

Damage potential and economic importance

CCNs can cause considerable yield losses, especially in semi-arid regions, temperate climates and monoculture systems (Rivoal and Cook, 1993). The damage threshold is determined by many environmental and genotypic factors such as nutrient availability and tolerant genotypes (Smiley and Nicol, 2009). It is difficult to compare studies of CCN economic damage, due to differences in sampling protocols, extraction procedures and enumeration.

H. avenae is a major limiting factor of wheat and barley; treatment of crops with nematicide in various countries has led to yield increases of 10–96% and 17–86% for wheat and barley, respectively (Holgado *et al.*, 2003; Namouchi-Kachouri *et al.*, 2007; Peng *et al.*, 2007; Hassan *et al.*, 2010). Less is known about the host range, biology and pathogenicity of *H. latipons*, but it is likely an important pest of barley and durum wheat in temperate, semi-arid regions (Philis, 1988; Sikora, 1988; Ismail *et al.*, 2001; Scholz, 2001; Scholz and Sikora, 2004; Al-Abed *et al.*, 2009). *H. filipjevi* is also economically important, due to its widespread distribution and previous misidentifications as *H. avenae* in the former USSR and Sweden.

Management

Managing CCNs is uniquely difficult, due to the eggs protected from potential parasites and rapid dessication inside the cyst (Riggs and Schuster, 1998). Eggs can remain dormant for many years; thus, management strategies must be effective for more than 1 year. The best management strategies are crop rotation and use of resistant cultivars, although chemical and biocontrol methods can also be considered.

CULTURAL PRACTICES: CCNs have a relatively narrow host range; thus, crop rotation is vital for their management. Although long rotations (4 years in Europe) have the potential to control CCNs, economics do not permit it in most subtropical and tropical countries. Since cereals are the prominent rotation crops in some countries, specific geographic knowledge of CCN species and pathotypes is necessary. For example, oat is resistant to CCNs in Australia, but susceptible in the UK (Cook and York, 1988).

Clean fallow and one to five deep ploughings during hot summer months can substantially reduce CCN population densities (Mathur *et al.*, 1987), but is not always economically and environmentally sound (Dababat *et al.*, 2015b). Other management strategies include: quarantine, cleaning machinery, field sanitation, weed control, organic and/or inorganic fertilizers, selecting sowing dates to escape J2 hatching peaks and trap cropping (Rivoal and Nicol, 2009; Smiley and Nicol, 2009; Dababat *et al.*, 2011; Dawabab *et al.*, 2015).

RESISTANCE: cultivar resistance is considered one of the best methods for nematode control and has been utilized successfully on farms in Australia, France, Sweden and Turkey (Rivoal and Nicol, 2009; Smiley *et al.*, 2013; Dababat *et al.*, 2014a, 2015b; Cui *et al.*, 2016; Pariyar, *et al.*, 2016b). However, the use of resistance, especially derived from single dominant genes, may cause disequilibrium in biological communities, and possibly ecological replacement with other nematodes such as *Pratylenchus* (Lasserre *et al.*, 1994; Turner and Rowe, 2006). Another potential concern is penalties in yield/quality or the breakdown of resistant germplasm (Turner *et al.*, 1983; Whitehead, 1998).

For the successful use of resistance, knowledge of the number of CCN species and pathotypes within species is essential. The International Cereal Test Assortment of barley, oat and wheat (Andersen and Andersen, 1982) offers classification of pathotype variation (Table 6.2). Although useful, a pathotype scheme for a species complex, based on the interaction with three cereal genera, will not easily describe extensive variation in virulence (Rivoal and Cook, 1993).

The extensive review by Rivoal and Cook (1993), revised by Nicol (2002) and presented in Table 6.3, indicates accessions of germplasm within oat, barley, triticale, rye, wheat and wild grass relatives that offer control of some CCN species and pathotypes and, where known, the genetic control and chromosome location. Some resistant cultivars simultaneously reduce CCN population densities of several European pathotypes (Williams and Siddiqi, 1972). Many of the sources implicate major gene inheritance, making selection for these relatively efficient.

Furthermore, many wild grass relatives have been introgressed into a hexaploid wheat background for breeding purposes. Nine resistance genes from different sources of *T. aestivum*, *Triticum tauschii*, *Aegilops ventricosa*, *Aegilops triuncialis* and *S. cereale* have been reported to confer total or partial resistance to different CCN pathotypes (Toktay *et al.*, 2012; Dababat *et al.*, 2015b). Some of these markers are used in marker-assisted selection (MAS) and pyramiding of gene resistance in Australian cereal breeding programmes against *H. avenae* (Ogbonnaya *et al.*, 1996; Jefferies *et al.*, 1997).

Table 6.3. Principal sources of genes^a used for wheat breeding resistance to cereal cyst nematode (*Heterodera avenae*) and root lesion nematode (*Pratylenchus thornei* and *Pratylenchus neglectus*).

Cereal species	Cultivar or line	Origin	Genetic information R-gene(s) ^b	Response to pathotypes ^c	Use in cultivars	References ^d
Cereal cyst nematode:						
Oat						
<i>Avena sterilis</i>	I376	?	1–3 major genes	R to all <i>Ha1</i> , <i>Ha2</i> and <i>Ha3</i>	UK	
<i>Avena</i> spp.	US1624 (CI3444)		Major gene	R to <i>Ha1</i> and <i>Ha2</i> , S to <i>Ha3</i>	Sweden, Denmark, UK	
<i>Avena</i> spp.	Avon and several Australian cvs.	?		R in Australia (<i>Ha13</i>), S to <i>Ha1</i> and <i>Ha2</i>	Australia	
<i>Avena sativa</i>	Panema Nelson	UK Sweden	1 dom, from I376 1 dom, from C.I. 3444, allelic to Panema	South Australia —	UK North-west Europe	
<i>Avena byzantina</i>	NZ Cape	New Zealand	2 dom	S, UK	France	
	Mortgage Lifter	Australia	? 2 rec	—	Australia	
	TAMO 301, 302	Texas, USA	?	—	Australia	
	No 11527	?	?	R, Siberia		
Barley						
<i>Hordeum</i> spp.	Many cvs.	Northern Europe landraces	<i>Rha1</i>	R, to <i>Ha1</i> in many cvs.	N. Europe cvs. 1900–1950s	
	Emir		<i>Rha?</i>	R to <i>Ha61</i> (Norway, NL, India, Siberia)	Susceptible in most of Europe	
	North African accessions?	N. Africa	<i>Rha2</i>	R to <i>Ha1</i> , <i>Ha2</i> and S to <i>Ha3</i>	Cvs. in Denmark, Sweden, UK	
	Morocco from North Africa	Morocco	<i>Rha3</i>	R to <i>Ha1</i> , <i>Ha2</i> and <i>Ha3</i>	Not in cultivars	
	Galleon		Major gene	R to <i>Ha13</i>	Australia	
	Drost	Sweden	1 dom (<i>Rha1</i>)	many bred cvs.		
	Ortolan	Germany	1 or 2 dom, allelic to <i>Rha1</i>	pR, Australia	N. Europe	

	ex. L.P.191 ex. Morocco L.P. 191, Morocco	?N. Africa N. Africa	1 dom, (<i>Rha2</i>) not linked to <i>Rha1</i> 1, dom, (<i>Rha3</i>) allelic to <i>Rha2</i> 1–2 dom		N. Europe Australia —	
	Athenais Nile, C.I.3576 C.I.8147 Martin C164, RD2052	Greece Egypt Turkey Algeria India	1 dom, not <i>Rha1</i> 1 dom, similar to <i>Rha2</i> 1 dom, not <i>Rha1</i> 2 dom, ?similar to <i>Rha3</i> 1 dom	R, pathotype-1 (Delhi population) India	Australia Australia Australia Australia	
Wheat						
<i>Triticum aestivum</i>	Loros, AUS10894	?	Cre1^a (formerly <i>Ccn1</i>), on chromosome 2BL	pR to several pathotypes	N.W. Europe, Australia	Slootmaker <i>et al.</i> (1974); Bekal <i>et al.</i> (1998)
	Katyil Festiguay	Australia Australia	Ccn1 Cre8 (formerly <i>CreF</i>) on chromosome 7L? Recent analysis suggests 6B	S, India pR in cvs. Australia (<i>Ha13</i>)	Australia Australia	Paull <i>et al.</i> (1998); Williams <i>et al.</i> (unpublished)
	AUS4930 = 'Iraq 48'	Iraq	Possibly identical genetic location to <i>Cre1</i> . Also resistance to <i>Pratylenchus thornei</i>	R to several pathotypes and CCN species and <i>Pratylenchus thornei</i>	Australia, France, CIMMYT Int. — under scientific evaluation	Bekal <i>et al.</i> (1998); Nicol <i>et al.</i> (1998, 1999, 2001); Green (personal communication); Lagudah (personal communication)
<i>Triticum durum</i>	Psathias 7654, 7655, Sansome, Khapli	?	?	S, to some patho- types, pR to others	—	Rivoal <i>et al.</i> (1986)
Triticale						
<i>Triticosecale</i>	T701-4-6	Australia	<i>CreR</i> on chromosome 6RL		Australia	Asiedu <i>et al.</i> (1990); Dundas <i>et al.</i> (2001)
<i>Secale cereale</i>	R173 family		<i>CreR</i> on chromosome 6RL			Taylor <i>et al.</i> (1998)
	Driva	Australia	?	= Ningadhu in cv. Tabara	Australia	
	Salvo	Poland	?		UK	

Continued

Table 6.3. Continued.

Cereal species	Cultivar or line	Origin	Genetic information R-gene(s) ^b	Response to pathotypes ^c	Use in cultivars	References ^d
Wild grass relatives						
<i>Aegilops tauschii</i>	CPI 110813	Central Asia	<i>Cre4</i> on chromosome 2DL	R Australia	Australia synthetic hexaploid lines	Eastwood <i>et al.</i> (1994); Rivoal <i>et al.</i> (2001)
<i>A. tauschii</i>	AUS18913	?	Cre3 on chromosome 2DL	R Australia	Australia advanced breeding lines	Eastwood <i>et al.</i> (1994); Rivoal <i>et al.</i> (2001)
<i>Aegilops peregrina</i> (<i>Aegilops variabilis</i>)	1		<i>Cre(3S)</i> with (<i>Rkn2</i>) on chromosome 3S; <i>CreX</i> , not yet located			Barloy <i>et al.</i> (1996); Jahier <i>et al.</i> (1998); Rivoal <i>et al.</i> (2001); Barloy (unpublished); Lagudah (personal communication)
<i>Aegilops longissima</i>	18	?	?	R to four French pathotypes and <i>Meloidogyne naasi</i>	France	Bekal <i>et al.</i> (1998)
<i>Aegilops geniculata</i>	79 MZ1, MZ61, MZ77, MZ124		?	R and pR to several pathotypes	France – under scientific evaluation	Bekal <i>et al.</i> (1998); Zaharieva <i>et al.</i> (2001)
<i>Aegilops triuncialis</i>	TR-353	?	<i>Cre7</i> (formerly <i>CreAet</i>)	R and pR to several pathotypes	France – under scientific evaluation	Romero <i>et al.</i> (1998)
<i>Aegilops ventricosa</i>	VPM 1		<i>Cre5</i> (formerly <i>CreX</i>), on chromosome 2AS	R to several pathotypes	Spain – under scientific evaluation	Jahier <i>et al.</i> (2001); Ogonnaya <i>et al.</i> (2001b)
	11, AP-1, H-93-8		<i>Cre2</i> (formerly <i>CreX</i>) on genome N ^v			Delibes <i>et al.</i> (1993); Andrés <i>et al.</i> (2001); Rivoal <i>et al.</i> (2001)
	11, AP-1, H-93-8, H-93-35		<i>Cre6</i> , on chromosome 5N ^v			Ogonnaya <i>et al.</i> (2001b); Rivoal <i>et al.</i> (2001)

Root lesion nematode <i>T. aestivum</i>	GS50a	Australia – reselection from Australian cultivar ‘Gatcher’	Resistance to Pt		Thompson and Clewett (1986)
	AUS4930 = Iraq 48	Iraq	Resistance to Pt but also portrays resistance to CCNs	Australia, CIMMYT – under scientific investigation	Nicol <i>et al.</i> (1998, 1999, 2001)
	Excalibur	Australia – reselection of commercial cultivar ‘Excalibur’	Resistance to Pn (<i>Rlnn1</i>), on chromosome 7AL		Williams <i>et al.</i> (2002)
	Croc_1/ <i>Ae. tausch.</i> (224)//Opata	Synthetic derivative	Resistance to Pt. Unknown from where resistance is derived		Nicol <i>et al.</i> (2001)
	<i>A. tauschii</i>		Resistance to Pt and Pn		Thompson (personal communication)
<i>A. geniculata</i>	MZ10, MZ61, MZ96, MZ144	Middle East and West Asia	Moderate resistance to Pt. Several also portray resistance to CCNs		Zaharieva <i>et al.</i> (2002)

Notes: ^aSee also differentials listed in Table 6.2. ^bdom or rec = dominant or recessive genes. Characterized single gene; bold indicates a marker implemented in a commercial breeding programme; see Ogbonnaya *et al.* (2001a,b). ^cR = resistant; pR = partially resistant; S = susceptible. ^dSourced from Rivoal and Cook (1993) and Cook and Rivoal (1998). Pt = *Pratylenchus thornei*; Pn = *Pratylenchus neglectus*; ? = no published scientific studies conducted.

Utilizing resistance sources is country specific and dependent on the crop and the number and pathotypes of *Heterodera* spp. For example, in Israel, all locally grown wheat and barley cultivars tested are excellent hosts of *H. avenae* and *H. latipons*, whereas oat cultivars are poor hosts of *H. avenae* but good hosts of *H. latipons* (Mor *et al.*, 1992). Many countries unfortunately have limited resources and/or expertise to generate this information, and current control methods are based on understanding the response of local cultivars to the pathogen(s). Greater collaborations are therefore essential between research institutions (e.g. Consultative Group on International Agricultural Research (CGIAR)) and countries where CCNs are problematic (Rivoal *et al.*, 2001).

CHEMICAL: low rates of non-fumigant nematicides provided effective and economical control under severe infestation conditions in nematode control programmes in Australia, India, Pakistan and Turkey (Gurner *et al.*, 1980; Sharma and Swarup, 1984; Maqbool, 1988; Dababat *et al.*, 2011, 2014b). However, the present-day cost and environmental concerns associated with these chemicals do not render them a viable economic option for most wheat farmers. This could change with the development of cheaper, safer and more effective seed-treatment nematicides (see Chapter 23, this volume), especially where no resistant cultivars are available (Dababat *et al.*, 2014a). The use of nematicides in research to understand the importance of these nematodes and new species as they are detected will, however, remain vital.

BIOLOGICAL CONTROL: biological control is an environmentally sensitive strategy with the potential to reduce nematode multiplication and subsequent damage to crops (Kerry, 2000; Ashoub and Amara, 2010; Viaene *et al.*, 2013), although it is difficult to generate nematode-suppressive soils to control CCN populations below economic damage thresholds. Most research on biological control has been conducted on obligate parasites, facultative parasites and rhizosphere bacteria (Dababat *et al.*, 2015b). Nematophagous fungi, such as *Nematophthora gynophila*, *Trichoderma longibrachiatum* and *Pochonia chlamydosporium*, were effective for managing *H. avenae*, *H. filipjevi* (Kerry *et al.*, 1982a,b; Holgado and Crump, 2003; Zhang *et al.*, 2014, 2015) and *H. latipons*

(Ismail *et al.*, 2001) populations. Species of *Streptomyces*, *Bacillus* and *Pasteuria*, and other bacteria, are also used to manage CCNs (Gokte and Swarup, 1988; Sharma and Swarup, 1988; Sayer *et al.*, 1991; Bansal *et al.*, 1999; Yavuzaslanoglu *et al.*, 2011; Zhang *et al.*, 2016a,b).

Studies conducted in the UK demonstrated that, despite cereal monocultures, *H. avenae* densities were naturally maintained below an economically damaging threshold by parasitic fungi (Kerry and Crump, 1977; Kerry, 1981; Kerry *et al.*, 1982a,b,c; Crump *et al.*, 1983; Dababat *et al.*, 2015b). Dackman and Nordbring-Hertz (1985) found that cysts of *H. avenae* in Sweden, in which all eggs were parasitized, commonly gave rise to pure colonies of egg parasites, while cysts in which only a portion of the eggs were infected gave rise to multiple opportunistic species. Sharma and Swarup (1988) identified a species of the nematode parasitic bacteria *Pasteuria* and suggested that it might be a promising agent for *H. avenae* control. The use of mutualistic fungal and bacterial endophytes to reduce nematodes is also promising (Sikora, 1992; Padgham and Sikora, 2007). A study by Ashrafi *et al.* (2015) showed that endophytes reduced *H. filipjevi* cyst densities by 50% in a wheat crop.

Several commercial biological control products are available for controlling sedentary nematodes, including cyst and root knot nematodes (see Chapter 23, this volume). These include Clariva®, a seed treatment used on soybean that contains the bacterial parasites *Pasteuria nishizawae*. In the USA and South Africa, seed coating with PONCHO®/VOTiVo® (or Poncho® Votivo®) is used as a biocontrol agent on maize in combination with fungicides and/or insecticides (Anon., 2016). Biological agents are more commonly used on higher-value cash crops (e.g. tomato) and seldom on cereal crops, especially wheat, where the cost of seed treatment is too high. The greatest value of biocontrol agents will be in combination with other control options (Trudgill *et al.*, 1992).

Root lesion nematodes: *Pratylenchus*

Distribution

The genus *Pratylenchus* constitutes many species affecting both monocots and dicots. Traditional

identification of *Pratylenchus* spp. relied on the morphological characteristics of the adult stages (Loof, 1991; Handoo *et al.*, 2001), but diversity in morphology and morphometrics among and within species is often reported, as a result of the host plant and/or varying environmental conditions (Doucet *et al.*, 2001). At least eight *Pratylenchus* spp. parasitize cereal roots (Rivoal and Cook, 1993), of which *Pratylenchus thornei*, *Pratylenchus crenatus*, *Pratylenchus neglectus* and *Pratylenchus penetrans* are distributed worldwide and sometimes coexist (Kort, 1972; Smiley and Nicol, 2009; Smiley, 2010). The geographic distribution of *Pratylenchus* spp. depends mostly on the prevalence of host plants and abiotic factors, especially temperature (Castillo and Vovlas, 2007).

P. thornei is the most studied species parasitizing cereals worldwide. It is found in Algeria, Australia, Canada, Iran, Israel, Italy, Mexico, Morocco, Pakistan, India, Syria, Turkey, the USA and former Yugoslavia (Greco *et al.*, 1984; Saxena *et al.*, 1988; Smiley *et al.*, 2004; Sheedy *et al.*, 2008; Smiley, 2010; Mokrini *et al.*, 2016). In the Pacific Northwest of the USA, *Pratylenchus* spp., dominated by *P. neglectus* and *P. thornei*, are present in over 90% of dryland wheat fields.

Biology and life cycle

Pratylenchus spp. are migratory endoparasites (Moens and Perry, 2009), and all their motile life stages are parasitic (Bridge and Starr, 2007). The *Pratylenchus* life cycle (see Chapter 2, this volume) ranges between 45 and 65 days, depending on the species, food availability, temperature, host species and moisture (Taylor *et al.*, 2000). *Pratylenchus* spp. can complete three to six generations within the roots during a single cropping season (Taylor *et al.*, 2000).

Environmental factors

P. thornei is active during the winter growing season in North Africa and survives the hot, dry summer period between wheat and the next crop in a desiccated state for up to 7–8 months, until reactivated by rainfall (Grandison and Wallace, 1974; Glazer and Orion, 1983). *P. crenatus* is more common in light soils, *P. neglectus* in loamy soils and *P. thornei* in heavier soils (Kort, 1972), although both *P. thornei* and *P. neglectus* can occur in a range of soil types, and mixtures

of the two species are not uncommon (Nicol, 1996; Nicol *et al.*, 2002).

Nitrogen, commonly applied to cereals, can have important effects on *P. thornei*. Van Gundy *et al.* (1974) reported nitrogen application to provide some level of control, but only when population levels neared the economic threshold for damage. However, Doyle *et al.* (1987) working with *P. thornei* and Orion *et al.* (1984) with *Pratylenchus mediterraneus* found no effect of nitrogen on nematode population dynamics. Kimpinski (1972) found that the concentration of ammonium nitrate was correlated with lower densities of *P. neglectus* in wheat roots. However, potassium and phosphorus fertilizers did not increase wheat yields significantly in *P. thornei*-infested fields (Doyle *et al.*, 1987) and caused no change in *P. neglectus* densities (Kimpinski, 1972).

Symptoms of damage

Generally, water-soaked lesions appear first on roots after *Pratylenchus* spp. penetrate the root epidermis. Such lesions may overlap, can reach 1–2 mm in length and change colour to green and reddish brown (Fig. 6.4) (De Waele and Elsen, 2002). *Pratylenchus* spp. cause degradation of epidermal and cortical cells of underground plant organs, reducing the amount of root branching and hairs, as well as degrading the lateral roots, ultimately reducing the plant's ability to access water and nutrients (Vanstone *et al.*, 1998; Smiley, 2010). Above-ground symptoms are non-specific, with infected plants appearing stunted with premature yellowing of older leaves, reduced tillering and lower weight (Castillo and Vovlas, 2007; Smiley, 2010). This often results in confusing the symptoms with nutrient deficiencies, drought, root disease, barley yellow dwarf disease or damage by other pathogens (Evans and Haydock, 1993; Taylor *et al.*, 1999; Smiley, 2010). Penetration of roots by lesion nematodes facilitates colonization by other pathogens, e.g. root-rotting fungi, saprophytic bacteria and non-parasitic nematodes, resulting in increased yield losses (Evans and Haydock, 1993; Moens and Perry, 2009; Smiley, 2010).

Pathotypes

Biological diversity has been observed among populations of *Pratylenchus brachyurus*, *Pratylenchus*

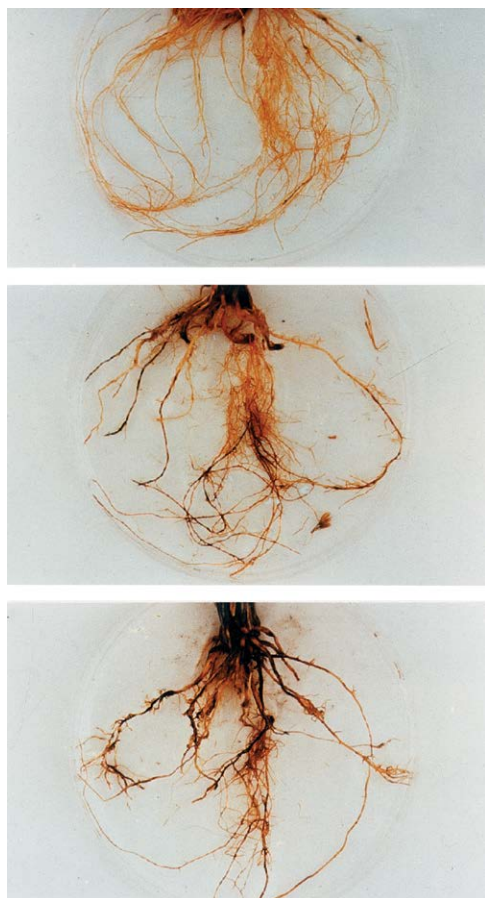


Fig. 6.4. Symptoms of root lesion nematode, *Pratylenchus thornei*, on wheat, showing extensive lesions, cortical degradation and reduction in both seminal and lateral root systems with increasing nematode density from top to bottom under natural field infestations. (Photograph courtesy of J. Nicol, CIMMYT.)

goodeyi, *Pratylenchus loosi*, *P. neglectus*, *P. penetrans* and *Pratylenchus vulnus* (De Waele and Elsen, 2002) but not within *P. thornei*. Screening of identified resistant wheat accessions in Australia, Mexico and Turkey with local populations revealed resistance to be stable. The reproductive fitness of root lesion nematode populations should be tested before using the population for resistance screening, since nematodes reared *in vivo* for an extended period can lose their pathogenicity (De Waele and Elsen, 2002).

Damage potential and economic importance

Pratylenchus spp. are considered the second most important nematode group of wheat, in terms of their potential for economic damage (Smiley *et al.*, 2005; Castillo and Vovlas, 2007; Keil *et al.*, 2009; Smiley and Nicol, 2009). Their impact on plant health and crop yield varies with location, cropping sequence and intensity, cultivar selection, soil characteristics and nematode community structure (McKenry and Ferris, 1983). *P. thornei* is the most studied on wheat and it is the most important (economically) species, with yield losses of 10–85% (Nicol and Ortiz-Monasterio, 2004; Smiley *et al.*, 2005; Thompson *et al.*, 2008; Toktay, 2008; Mokrini *et al.*, 2009).

P. neglectus and *P. penetrans* do not have the same level of global distribution or damage potential as *P. thornei*. Wheat yield losses due to *P. neglectus* range from 10 to 23% (McDonald and Nicol, 2005) or 56 to 74% when both *P. thornei* and *P. neglectus* are present (Vanstone *et al.*, 1998).

Molecular identification and quantification

Traditional identification of *Pratylenchus* spp. is being supported increasingly with molecular data, since DNA-based methods provide efficient tools for precise and rapid identification (Waeyenberge *et al.*, 2000; Subbotin *et al.*, 2006). PCR, using species-specific primers of *Pratylenchus*, constitutes a major step forward in the development of diagnostic technology. Species-specific primers to detect *P. penetrans* in a conventional PCR have also been developed (Al-Banna *et al.*, 2004; Waeyenberge *et al.*, 2009). Quantitative PCR (qPCR) tools have also been developed for *P. zeae* (Berry *et al.*, 2008), *P. penetrans* (Mokrini *et al.*, 2013), *P. thornei* (Yan *et al.*, 2012; Mokrini *et al.*, 2014) and *P. neglectus* (Yan and Smiley, 2013).

Management

Several countries (e.g. Australia, Israel, Mexico, Turkey) utilize integrated pest management strategies to reduce *Pratylenchus* densities below threshold levels. Damage thresholds (Table 6.4)

Table 6.4. Damage threshold densities of *Pratylenchus*–cereal combinations.

<i>Pratylenchus</i> spp.–cereals	Damage threshold (nematodes/cm ³ soil)	Reference
<i>Pratylenchus crenatus</i> –oat	0.33	Barker and Olthof (1976)
<i>Pratylenchus neglectus</i> –barley	1.5	Rivoal and Cook (1993)
<i>Pratylenchus thornei</i> –wheat	0.5–1	Rivoal and Cook (1993)
<i>P. thornei</i> –wheat	0.42	Nicol and Ortiz-Monasterio (2004)
<i>P. thornei</i> –wheat	2.5	Thompson (1993)
<i>P. thornei</i> –wheat	3	Nicol <i>et al.</i> (1999)

vary among *Pratylenchus* spp. and crops, and depend on geographic location, crop value and the occurrence of disease complexes (Davis and MacGuidwin, 2000; Castillo and Vovlas, 2007). Potential crop damage caused by *Pratylenchus* is usually based on soil densities at planting, but also on densities in roots during the growing season. The choice of management depends on accurate diagnosis of the species and population levels.

CULTURAL PRACTICES: field sanitation during the fallow phase is crucial because *Pratylenchus* can multiply on many weed species between wheat crops (Vanstone and Russ, 2001; Smiley *et al.*, 2004). Crop rotation has limited effectiveness against root lesion nematodes, due to their polyphagous nature (Nicol and Rivoal, 2008). However, some effective rotations have been developed to reduce *P. thornei* numbers in wheat fields, for example, in Sonora (Mexico) using maize, cotton or soybean rotations for 2 consecutive years, and in Queensland (Australia) using barley cv. Clipper. Triticale cvs. Abacus and Muir host fewer *Pratylenchus* than bread or durum wheats, offering other useful rotational options (McDonald and Nicol, 2005).

RESISTANCE: the use of resistant and tolerant cultivars is the most economical and environmentally acceptable method of controlling *Pratylenchus* spp. (Castillo *et al.*, 1998). Tolerant wheat cultivars, such as Pelsart (Brennan *et al.*, 1994), Sunvale (Ellison *et al.*, 1995) and Baxter (Thompson *et al.*, 1999) offer a 30% yield increase in infested fields compared to commercial cultivars (Thompson *et al.*, 1995). On resistant cultivars, nematode reproduction is reduced, while a tolerant cultivar has the capacity to grow and yield well in the presence of high *Pratylenchus*

numbers but do not reduce their numbers in the soil (Thompson *et al.*, 1999).

Resistance against *P. thornei* has been studied more in wheat than for *P. neglectus*, since the former is more damaging in northern Australian wheat systems (Thompson *et al.*, 2008). As both *Pratylenchus* spp. are often found in mixed populations (Thompson *et al.*, 2010), it is desirable to develop wheat cultivars with resistance to both. The soil-borne pathogen programme at CIMMYT Turkey annually screens about 1000 accessions of wheat from the Turkey-CIMMYT-ICARDA International Winter Wheat Improvement Program (www.iwwip.org) and the CIMMYT Mexican spring wheat breeding programme for multiple disease resistance, including for *P. thornei* and *P. neglectus* (Toktay *et al.*, 2012; Dababat *et al.*, 2016). The first source of resistance to *P. thornei* was the bread wheat line GS50a, selected from healthy plants within the susceptible cv. Gatcher (Thompson and Clewett, 1986). GS50a resistance was subsequently backcrossed into commercial wheat cultivars that reduced *P. thornei* reproduction more than tenfold compared to susceptible cultivars (Thompson *et al.*, 1999).

Molecular markers for resistance to both *P. thornei* and *P. neglectus* have also been developed (McIntosh *et al.*, 2001; Toktay *et al.*, 2012). *Rln1*, the first resistance gene for *Pratylenchus* spp. to be mapped, is located on chromosome 7A (Williams *et al.*, 2002).

CHEMICAL: chemical control is effective against *Pratylenchus* in most cases, but it is not generally economical nor environmentally acceptable for cereal crops.

BIOLOGICAL CONTROL: successful biological control of *Pratylenchus* spp. is likely to be difficult

due to the nematodes' migratory behaviour (Timper and Brodie, 1994). Many biological control organisms may kill lesion nematodes (Timper and Brodie, 1993); however, few studies have evaluated these organisms under field conditions. In soils of grain production areas in northern Australia, several antagonists of lesion nematodes have been identified, such as endophytic fungi, nematode trapping fungi, predatory nematodes and parasitic bacteria (Seymour *et al.*, 2016).

Seed gall nematode: *Anguina tritici*

Distribution

A. tritici was the first plant parasitic nematode described. It is commonly known as 'ear cockle' and is frequently found in seeds of small grain cereals where retained seed is sown without using proper cleaning systems. It infects cereals throughout western and South Asia, North Africa, Europe, Australia, Brazil, China, Pakistan, Russia, New Zealand and several areas in the USA (cited in McDonald and Nicol, 2005; Mohamedova and Piperkova, 2013; Tulek *et al.*, 2015).

Biology and life cycle

Seed gall nematodes are amphimictic; females lay eggs inside the seeds that form galls containing infective but quiescent J2. During sowing, galls reach the soil and become moist and soft, facilitating the release of juveniles. On average, a single wheat seed gall contains 11,790 dormant juveniles (Tulek *et al.*, 2015). Approximately 1 week after seed galls are sown, juveniles can be found in the growing point of the germinating plant. These juveniles move passively upward on the growing point as the plant grows. They do not exhibit any morphological changes until they penetrate a flower primordium (2–3 months after sowing) and become adults. Ovules and other flowering parts of a plant are then transmuted into galls or 'cockles' (Figs 6.5 and 6.6). Nematodes mature inside galls and females lay thousands of eggs, from which juveniles hatch but remain dormant within the seed. One generation is completed per crop season and the total life period duration is 106–113 days (Swarup and Sosa-Moss, 1990). Temperature, humidity,



Fig. 6.5. Wheat ear head with ovules of wheat infected with *Anguina tritici* with exposed swollen black seed galls, or 'cockles'. (Photograph courtesy of A. Tulek and A. Dababat.)

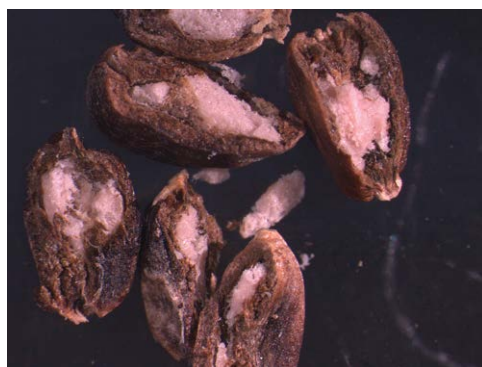


Fig. 6.6. Mature *Anguina tritici* black seed galls, or 'cockles', on wheat seed. (Photograph courtesy of A. Tulek and A. Dababat.)

planting depth and gall source are the major determinants in symptom expression. The nematode favours wet and cool weather (Kort, 1972).

Temperature, humidity and the source of galls are particularly important for the development of yellow ear rot (Fig. 6.7), a nematode-vectored bacterial disease commonly associated with the seed gall nematode. The disease was first recorded in India (Hutchinson, 1917), where the nematode was associated with the bacterium *Corynebacterium michiganense* pv. *tritici*. Alone, the bacterium results in yellow streaking of the leaves only, which run parallel to the veins. The bacterium multiplies very quickly under favourable environmental conditions, forming a thick, viscous fluid in which nematode juveniles are not able to survive. Under such conditions, emerging ears are totally

sterile. Under conditions that are less favourable for the bacterium, nematode juveniles survive to produce partial ear cockle and partial yellow ear rot symptoms. Economic losses associated with this complex are perpetuated because of the lower market price for infected grain (Rivoal and Cook, 1993).

Symptoms of damage

General symptoms of *A. tritici* infection are small and dying plants with twisted leaves (Swarup and Sosa-Moss, 1990). Infected ears are recognized easily by their smaller size and darkened colour compared to normal seeds, but infected seeds may be confused easily with bunt (*Tilletia tritici*). However, bunt balls give off a foetid or

fishy odour when crushed, whereas a cut or crushed seed gall reveals a white mass of nematode bodies. Under dry conditions, juveniles may survive for decades (Kort, 1972).

In both ear cockle and yellow ear rot, the first symptom is an enlargement of the basal stems of 3-week-old wheat seedlings. Emerging leaves are twisted (Figs 6.8 and 6.9) and crinkled. Some leaves may remain folded, with their tips held near the growing point; after 30–45 days, plants may straighten and appear normal, with faint ridges on the surface. Symptoms are more clearly discernible on young seedlings, and decrease with plant age. Under very low infection levels, plants may not exhibit any visible symptoms, even though a few seed galls occur in the ears. Infected seedlings produce more tillers and grow faster than normal plants, but do not necessarily have more ears (Swarup and Sosa-Moss, 1990). Ears emerge roughly 1 month earlier in diseased plants, are short and broad, with very small or no awns on the glumes. Nematode galls replace either all or some of the grains. In yellow ear rot disease, the characteristic feature is a bright yellow slime- or gum-like substance produced on abortive ears and leaves, which remains in contact with such ears while still in the boot-leaf stage. Under humid conditions, the bacterial slime trickles down the tissues (Swarup and Sosa-Moss, 1990), becoming brown on drying. An infected spike is narrow and short, with wheat grains replaced partially or completely by slime, with emerging spikes remaining sterile. The stalks of infected spikes are always distorted.



Fig. 6.7. *Anguina tritici* infection of wheat in India, with symptoms of 'yellow ear rot' caused by the interaction of the nematode with *Corynebacterium michiganense* pv. *tritici*. (Photograph courtesy of R. Sikora.)



Fig. 6.8. *Anguina tritici* induced enlargement of basal stems of 3-week-old wheat seedlings and typical crinkling and twisting of emerging leaves. (Photograph courtesy of A. Tulek and A. Dababat.)



Fig. 6.9. *Anguina tritici* infection on 30- to 45-day-old plants showing crinkled edges with necrosis along leaf edges. (Photograph courtesy of A. Tukul and A. Dababat.)

Damage potential and economic importance

Worldwide, *Anguina* attacks wheat, barley and rye. In association with yellow ear rot bacterium, *Anguina* can seriously affect wheat yields (Maqbool, 1988), causing losses of up to 30% in Iraq (Stephan, 1988), 10–30% in China (Chu, 1945) and 51–60% under artificial inoculation (Tulek *et al.*, 2015). Disease intensity is greatest when nematode galls are sown at a depth of 2–6 cm, rather than deeper.

Management measures

SEED HYGIENE: ear cockle can be controlled easily by seed hygiene, using either certified seed or seeds that have been ‘cleaned’ (Singh and Agrawal, 1987). These techniques have enabled *Anguina* to be eradicated from the western hemisphere, although it remains problematic in western and South Asia, and to some extent China (Swarup and Sosa-Moss, 1990).

Since seed galls are the only source for perpetuation of both ear cockle and yellow ear rot, their removal can completely eliminate both diseases. Galls are lighter in weight than wheat

seed and can be removed easily by winnowing or by floating contaminated seeds in a 20% brine solution. Wheat seed should, however, be washed with water after brine treatment to remove adhering salt particles, otherwise seed germination is impaired.

RESISTANCE AND ROTATIONS: where hygiene practices are difficult to implement, host resistance and rotation offer some hope. Resistance to *A. tritici* has been identified in India, Iraq and Pakistan (McDonald and Nicol, 2005). Oat, maize and sorghum are considered non-hosts of *Anguina* (Limber, 1976; Paruthi and Gupta, 1987), but do not completely control the disease.

Root knot nematodes: *Meloidogyne* spp.

Distribution

Meloidogyne spp. are economically the most important group of plant parasitic nematodes worldwide. However, with the exception of the most important species, our knowledge of many remains limited, even though they attack most cultivated crops (Jones *et al.*, 2013). Several *Meloidogyne* spp. attack cereals; in warm climates, *Meloidogyne graminicola*, *Meloidogyne graminis*, *Meloidogyne kikuyensis* and *Meloidogyne spartinae* are important (Taylor and Sasser, 1978), whereas *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne arenaria* are most important in tropical and subtropical areas (Swarup and Sosa-Moss, 1990).

M. naasi (barley root knot nematode) occurs primarily in temperate climates and is probably the most important root knot nematode affecting grain in most European countries (Kort, 1972). It has been reported in most northern European countries, the USA, Canada and the former USSR (Bélair *et al.*, 2006). However, it has also been found in Chile, Iran, the Maltese islands, New Zealand and Turkey (Kort, 1972; Inserra *et al.*, 1975; Jepson, 1987). *M. naasi* does not seem to be widespread in temperate, semi-arid regions such as western Asia and northern Africa (Sikora, 1988). In Europe, oat is a poor host compared with other cereals, whereas in the USA, oat is an excellent host of *M. naasi* (Kort, 1972).

Meanwhile *Meloidogyne artiellia* (British root knot nematode) is known to reproduce well

on cereals and severely damage legumes (Kyrou, 1969; Sikora, 1988). This nematode occurs in Algeria, France, Greece, Israel, Italy, Morocco, Russia, Spain, Syria, Tunisia, Turkey and the UK (Shiabova, 1981; Mamluk *et al.*, 1983; Di Vito and Zacheo, 1987; Sikora, 1988; Mor and Cohn, 1989; Imren *et al.*, 2014). In South Asia, populations of *M. graminicola* cause severe damage to wheat roots in rice–wheat rotations (Gaur and Sharma, 1999; Padgham *et al.*, 2004).

Meloidogyne chitwoodi is a pest on cereals in the Pacific Northwest of the USA and is also found in Australia, Mexico and South Africa (Eisenback and Triantaphyllou, 1991). Its hosts include many cereals (e.g. wheat, oat, barley and maize) and several dicots (Santo and O'Bannon, 1981). *M. graminis* is not known to be widely distributed; it is limited to the southern USA, where it is associated with cereals and turf grasses (Eriksson, 1972), although it has also been reported from coastal dunes in Germany and the Netherlands (<http://nematode.unl.edu/melgrami.htm>, accessed 13 November 2017).

Biology and life cycle

Meloidogyne spp. cause typical, small-sized root galls (Fig. 6.10). Egg masses attached to the posterior end of protruding females are normally transparent, but darken on exposure to air and can resemble *H. avenae* cysts. Young *M. naasi* juveniles invade cereal roots within 30–45 days of germination, after which small galls on root tips can be observed. *M. naasi* generally has one generation per season (Rivoal and Cook, 1993), although in warmer regions on perennial or volunteer grass hosts, more generations are possible (Kort, 1972). Eggs have a diapause, broken by increasing temperature following a cool period (Antoniou, 1989). Juveniles develop and females become almost spherical in shape. Females deposit eggs in an egg sac, which usually appears 8–10 weeks after sowing, embedded in the gall tissue (Kort, 1972). Large galls may contain 100 or more egg-laying females (Rivoal and Cook, 1993).

Symptoms of damage

Towards the end of a growing season, galling of the roots, especially the root tips, is common (Fig. 6.10). Galls are typically curved, horseshoe- or spiral-shaped (Kort, 1972). Symptoms of



Fig. 6.10. Roots of wheat with severe galling caused by *Meloidogyne naasi*. (Photograph courtesy of D. Coyne.)

M. naasi closely resemble those caused by *H. avenae*, with patches of poorly growing, yellowing plants that may vary in size from a few square metres to larger areas. Other root knot nematodes probably produce similar symptoms, but most are less studied than *M. naasi*.

Damage potential and economic importance

Information on the economic importance of root knot nematodes on cereals is limited to a few species. *M. naasi* can seriously affect wheat yields in Chile (Kilpatrick *et al.*, 1976) and Europe (Person-Dedryver, 1986). It has also caused significant barley yield losses in Belgium, France, the Netherlands, the UK and the USA (Allen *et al.*, 1970; Caubel *et al.*, 1972; Gooris and D'Herde, 1977; York, 1980), although it is not known to be widespread in temperate semi-arid regions (Sikora, 1988).

Wheat damage by *M. artiellia* has been recorded in Greece, Israel and Italy (Kyrou, 1969; Di Vito and Greco, 1988; Mor and Cohn, 1989). *M. chitwoodi*, an important potato pathogen, is recorded damaging cereals in Mexico and the

USA (Inserra *et al.*, 1985; Cuevas and Sosa-Moss, 1990). In controlled laboratory studies, *M. javanica*, *M. chitwoodi* and *M. incognita* have been shown to reduce wheat growth (Abdel Hamid *et al.*, 1981; Roberts *et al.*, 1981; Sharma, 1981; Nyczepir *et al.*, 1984), with *M. incognita* a known problem for wheat in north-western India (Swarup and Sosa-Moss, 1990).

Management measures

Control methods for root knot nematodes have been investigated primarily for *M. naasi*, the most economically important species. Management options include rotations with non-host crops, use of fallow during the hatching period (Allen *et al.*, 1970; Gooris and D'Herde, 1972), or use of resistant cultivars (Cook *et al.*, 1986; Bridge and Starr, 2007). Partial resistance has been found in barley and also in *Triticum squarrosa* and *Triticum monococcum*, while full resistance was identified in *Hordeum chilense*, *Hordeum jabatum*, *Triticum umbellulatum* and goat grass, *Aegilops variabilis* (= *Triticum variabile*) (Cook and York, 1982; Roberts *et al.*, 1982; Person-Dedryver and Jahier, 1985). Resistance has also been expressed in *H. chilense* (Person-Dedryver *et al.*, 1990; Yu *et al.*, 1990).

Rotations also offer some options for *M. artiellia*, as cowpea, lupin, sainfoin and maize may be useful non-hosts (Di Vito *et al.*, 1985). The use of biological agents, such as the nematophagous fungus *Purpureocillium lilacinum* (= *Paecilomyces lilacinus*), and predators, such as the trapping fungus *Monacrosporium lysipagum* and *Seinura paratenuicaudata* nematode may control both *Anguina* and *Meloidogyne* (Vats *et al.*, 2004; Khan *et al.*, 2006).

Stem nematodes: *Ditylenchus* spp.

Distribution

The genus *Ditylenchus* comprises many species that are prevalent in a wide range of climatic conditions from temperate, subtropical to tropical regions, where moisture regimes enable nematode infection, multiplication and dispersal (Plowright *et al.*, 2002). *D. dipsaci* is the most common and important species of stem nematode on cereals, particularly on oat and rye, and is

widespread throughout Africa, western and Central Europe, Argentina, Australia, Brazil, Canada and the USA (Plowright *et al.*, 2002).

Biology and life cycle

D. dipsaci is a migratory endoparasite that invades cereal foliage and the base of stems, where it migrates through tissues and feeds on adjacent cells. Reproduction continues inside the plant almost year-round, but is minimal at low temperatures. When an infected plant dies, nematodes return to the soil and infect neighbouring plants. Typical symptoms of stem nematode attack include basal swellings, dwarfing and twisting of stalks and leaves, shortening of internodes and many axillary buds, producing an abnormal number of tillers to give a plant a bushy appearance (Fig. 6.11). Heavily infected plants may die in the seedling stage, resulting in bare patches in a field, while other infected plants fail to produce flower spikes (Kort, 1972).



Fig. 6.11. Stem nematode, *Ditylenchus dipsaci*, damage on oats, showing severe dwarfing, twisting of leaves and an abnormal number of tillers, giving a plant a bushy stunted appearance. (Photograph courtesy of S. Taylor.)

The nematodes are highly motile in soil and can cover 10 cm in 2 h (Kort, 1972). There are more than 30 races/strains of *D. dipsaci*, which are morphologically indistinguishable but differ in host range (Janssen, 1994). Rye strains attack rye and oats, but also bean, maize, onion, tobacco, clover and a number of weed species commonly associated with cereals (Kort, 1972). The oat strain attacks oat, onion, pea, bean and several weed species, but not rye (Kort, 1972).

Damage potential and economic importance

Economic damage by *D. dipsaci* depends on many factors, such as host-plant susceptibility, soil infection levels, soil type and weather conditions, and is complicated by the extensive intra-specific variation among *D. dipsaci* races. Economic damage is rarely associated with sandy soils and is more likely in clay-based soils (Kort, 1972), posing problems for cereals cultivated in heavy soils in high rainfall areas (Griffin, 1984).

The nematode is economically important on rye and oat, but not on wheat and barley (Sikora, 1988).

Management measures

Management of *D. dipsaci* is difficult, due to its polyphagous nature and occurrence of various races/strains. Currently, the only economical and highly effective method is via host resistance (Table 6.5). *D. dipsaci* is hosted by lucerne, red and white clover, pea, bean and bulbous species of the Liliaceae, including garlic, onion, tulip and narcissus. Hence, the use of crop rotation is limited, except where resistant cultivars of these crops are used (Plowright *et al.*, 2002).

Wild oat, *Avena ludoviciana*, has multiple resistance genes (Plowright *et al.*, 2002), and several other oat cultivars are reported to express resistance or partial resistance/tolerance (Whitehead, 1998). In Britain, oat resistance derived from landrace cv. Grey Winter and controlled by a single dominant gene has been

Table 6.5. Crop cultivars and accessions resistant to stem nematode, *Ditylenchus dipsaci*. (From Plowright *et al.*, 2002.)

Crop	Species	Cultivar/accession	Country	Reference
Lucerne	<i>Medicago sativa</i>	Vertus Nova Washoe Lahontan Resistador II	Sweden Australia USA	Cook and Yeates (1993)
White clover	<i>Trifolium repens</i> Tolerant	Line G49	New Zealand	Mercer and Grant (1995)
		Sebeda Katrina Alice Donna Aran Pronitro	New Zealand UK	West and Steele (1986) Cook and Evans (1988)
Rye	<i>Secale cereale</i>	Ottersum (landrace) Heertvelder	The Netherlands	Ritzema-bos (1922)
Faba bean	<i>Vicia faba</i>	INRA 29H Several	France	Caubel and Le Guen (1983) Gastel (1990); Hanounik <i>et al.</i> (1986)
		Souk el Arba Rharrb (landrace)	Morocco	Schreiber (1977)
Red clover	<i>Trifolium pratense</i>	Sabtoron Norseman	UK	Plowright <i>et al.</i> (2002)
Oat	<i>Avena sativa</i>	Grey Winter Penirth Anita Bettong	Belgium Australia	Clamont (1985) MacDaniel and Barr (1994)
	<i>Avena ludoviciana</i>	Cc 4346	UK	Griffiths <i>et al.</i> (1957)

introgressed into several commercial cultivars (Plowright *et al.*, 2002).

Rotation of non-hosts, including barley and wheat, offers some control for the rye and oat races of *D. dipsaci*. However, once susceptible oat crops have been damaged, rotations are largely ineffective (Rivoal and Cook, 1993).

Other nematodes

Other nematode pests, e.g. *Longidorus elongatus*, *Merlinius brevidens*, *Tylenchorhynchus* spp. and *Nanidorus/Paratrichodorus* spp., also have the potential to cause yield loss on cereals, but their global distribution and damage potential have yet to be elucidated. *Tylenchorhynchus nudus*, *Tylenchorhynchus vulgaris* and *M. brevidens* are responsible for poor growth in parts of India and USA. Cereal crops in the USA are also subject to damage by *Paratrichodorus anemones* and *Paratrichodorus minor* (now known as *Nanidorus minor*) with wheat sown early in autumn in sandy soils being highly susceptible to *P. minor*.

Maize

Maize (*Zea mays*) is one of the most important cereal crops for both human consumption and livestock feed. Average total global maize production exceeds 900 Mt, outranking both paddy rice and wheat for the period 2010–2014 (<http://www.fao.org/faostat/en/#data/QC>). The USA is the biggest producer, followed by Asia (30%), South America (12%) and Africa (8%) (see Table 6.1).

The introduction of genetically modified (GM) maize expressing the *Bacillus thuringiensis* (Bt) gene, and the introduction of herbicide resistance to glyphosate, has revolutionized maize production. Information on the impacts of GM maize on non-target soil nematodes (plant parasitic and non-parasitic), while scarce, has shown no adverse effects on soil nematodes in Slovakia, the UK and the USA (Saxena and Stotsky, 2001; Griffiths *et al.*, 2005, 2007; Čerevková and Cagán, 2015).

Plant parasitic nematodes cause considerable loss during crop development, which is

intensified under drought and other stress conditions (Coyne *et al.*, 2009). Previously, maize was regarded as a non-/poor/immune host to several nematode species, probably because yield losses were not noticed due to extensive root systems, inadequate control measures (Riekert, 1996; Koenning *et al.*, 1999) or lack of typical symptoms (Asmus *et al.*, 2000; McDonald and Nicol, 2005; Bridge and Starr, 2007). However, the prominence of maize in world economies (see Table 6.1) highlights the potential impact of nematode parasitism, while the extensive use of maize in rotation systems necessitates knowledge of the crop's host status to important nematode pests.

Nematodes of Maize

Numerous nematode species are associated with maize across the world, but information on their biology and pathogenicity is generally scarce (Bridge and Starr, 2007). The most economically important plant parasitic nematode genera globally are: (i) root knot (*Meloidogyne*); (ii) root lesion (*Pratylenchus*); and (iii) cyst (*Heterodera*). *Pratylenchus*, *Meloidogyne* and *Hoplolaimus* are the most frequently reported genera on maize in the USA (Koenning *et al.*, 1999), while *Meloidogyne* and *Pratylenchus* are most common in South Africa (McDonald *et al.*, 2017). Other important nematode pests of maize are *Aphelenchoides* spp., *Paratrichodorus/Nanidorus* spp., *Longidorus brevianmulatus* and *Punctodera chalcensis* (Bridge and Starr, 2007; Nicol *et al.*, 2011; Mary *et al.*, 2013).

Meloidogyne

Distribution

The genus *Meloidogyne* (root knot nematodes) has close to 100 species and is considered economically important on most crops worldwide (Jones *et al.*, 2013). Some species have a worldwide distribution with wide host ranges, while others are limited in distribution and are more host specific. Several races with differential host ranges occur within species (Sasser and Triantaphyllou, 1977; Kleynhans, 1991). *M. incognita*

and *M. javanica* damage maize in almost all production regions, while *Meloidogyne africana* and *M. arenaria* occur in India and Pakistan. *M. arenaria* has also been reported in the USA and elsewhere (Kleynhans *et al.*, 1996; Davis and Timper, 2000; Timper *et al.*, 2002; Agenbag, 2016). *Meloidogyne enterolobii* is not regarded as a constraint to maize production, due to its poor/non-host status (Rosa *et al.*, 2012; EPPO, 2014).

Biology and life cycle

The life cycle of *Meloidogyne* spp. (see Chapter 2, this volume) can be completed within 3 weeks (Karssen, 2002) and is influenced predominantly by temperature, but also by abiotic and biotic conditions (Karssen *et al.*, 2013). Depending on the host plant and environmental conditions, one female can produce 30–80 eggs/day (Karssen *et al.*, 2013). *Meloidogyne* spp. thrive in soils with moisture levels at 40–60% field capacity (Karssen *et al.*, 2013). Under water stress, population densities of *M. incognita* increased in the roots of most inbred maize hybrids (Kagoda *et al.*, 2015).

Symptoms

Above-ground symptoms are generally rare or inconspicuous but include stunting, leaf chlorosis and patchy growth (Fig. 6.12). During extreme drought conditions in South Africa, stunted maize plants infected with *Meloidogyne* spp. showed phosphate deficiencies, visible as purple discoloration of leaf edges (McDonald *et al.*, 2017). Below-ground symptoms generally consist of root galling, which may be inconspicuous or distinct, and/or stunting of roots (Fig. 6.13). In heavily infected roots, females are visible where secondary roots emerge from main roots (Fig. 6.14).

Histologically, infection of maize roots by *M. javanica* shows typical multinucleated giant cell development in vascular tissue and embedded egg masses in inconspicuous galls, mostly close to root apices (Asmus *et al.*, 2000). Root galls are often small or lacking, so should be stained and examined if *Meloidogyne* is suspected or if J2 are detected in the soil samples. Root tip galls (Fig. 6.15) can also be confused with galls produced by ectoparasites such as *Xiphinema* (Fig. 6.16) or with aluminium toxicity



Fig. 6.12. Field damage showing stunted and sparse stand of maize caused by *Meloidogyne* spp. (Photograph courtesy of H. Fourie.)



Fig. 6.13. Root damage with inconspicuous root galling and branching caused by *Meloidogyne* on maize roots. (Photograph courtesy of A.H. McDonald.)



Fig. 6.14. Heavily infested maize roots with massive *Meloidogyne* spp. galling and associated root rotting. (Photograph courtesy of A.H. McDonald.)

(H. Fourie, Potchefstroom, South Africa, 2016, personal communication).

Meloidogyne assessments on maize include NaOCl extraction techniques, gall indices and staining methods (McDonald and Nicol, 2005). Use of the adapted NaOCl method (Riekert, 1995) leads to the extraction of *Rotylenchulus parvus* and *Meloidogyne* eggs from maize roots (Bekker *et al.*, 2016), with the genera confirmed using molecular tools.

Pathotypes

Populations of *M. incognita* and *M. arenaria* sometimes reproduce well on maize, but some cultivars exhibit specificity to a certain population

(McDonald and Nicol, 2005). The discovery of additional host races of *M. arenaria*, *M. incognita* and *M. javanica* from Spain illustrates the importance of screening maize against as many populations as possible (Robertson *et al.*, 2009).

Damage potential and economic importance

In South Africa, single- or mixed-species populations of *M. incognita* or *M. javanica* caused maize yield losses of up to 60% (Riekert, 1996; Riekert and Henshaw, 1998). Similarly, in Nigeria, *M. incognita* caused significant reductions in ear mass (64%) and grain yield (56%) (Odeyemi *et al.*, 2011). Commercial tropical hybrids are equally susceptible to *M. arenaria* and *M. incognita*

(Windham and Williams, 1994a; Davis and Timper, 2000).

Indirect observations after nematicide application in *Meloidogyne*-infested soils accentuate the economic importance of these pests in maize. Tolerance in maize to root knot nematodes is most probably due to extensive root growth after the seedling stage, and high fertilizer application and watering levels (McDonald and Nicol, 2005). Optimal reproduction of *Meloidogyne* spp. on

maize roots often results in damage of succeeding crops that are susceptible/less tolerant to these pests (Bridge and Starr, 2007).

Pratylenchus

Distribution

Lesion nematodes are cosmopolitan in maize fields and are often associated with poor growth and yield reductions (Landi and Manachini, 2005; McDonald and Nicol, 2005; Hassan *et al.*, 2009). *P. brachyurus*, *Pratylenchus zae* and *P. penetrans* are the most common species in subtropical and tropical maize production regions, while *Pratylenchus coffeae*, *Pratylenchus delattrei*, *P. goodeyi*, *Pratylenchus hexincisus*, *P. neglectus*, *Pratylenchus pratensis*, *Pratylenchus sefaensis*, *P. thornei*, *Pratylenchus sativus* and *Pratylenchus scribneri* have also been reported (McDonald and Nicol, 2005; Bridge and Starr, 2007; Hassan *et al.*, 2009; Olowe, 2011; Qiu *et al.*, 2016).

Biology and life cycle

The general biology and life cycle of lesion nematodes are described in Chapter 2, this volume. Temperature greatly affects the development and reproduction of *Pratylenchus*; for example, *P. zae*, *P. brachyurus* and *P. hexincisus* reproduce well at 30°C, whereas *P. penetrans* prefers lower temperatures (20–24°C). The optimum temperature for nematode development is frequently correlated with the optimum temperature required for optimal plant growth (McDonald and Nicol, 2005).



Fig. 6.15. Root tip galls on young maize plants caused by *Meloidogyne incognita* in South Africa. (Photograph courtesy of S. Bekker.)



Fig 6.16. Root tip galls and stubby root symptoms caused by *Xiphinema* spp. on maize. (Photograph courtesy of B. Jacobsen.)

Soil type and tillage practices also affect lesion nematode population densities. Most *Pratylenchus* spp. thrive in a wide range of soil types, although they generally favour coarsely textured sandy soils (McDonald and Nicol, 2005; Griffiths *et al.*, 2006; Bridge and Starr, 2007). Moisture is also an important factor affecting the development and damage potential of *Pratylenchus* spp. (McDonald and Nicol, 2005), although studies from Nigeria have reported increases in nematodes under both excessive moisture and water stress (Egunjobi, 1974; Kagoda *et al.*, 2015).

Symptoms of damage

Symptom expression generally depends on the nematode species, population density and environmental conditions; thus, above-ground symptoms are not highly specific (Jepson, 1987), although irregular clusters of *Pratylenchus*-infected plants may be visible in fields (Bridge and Starr, 2007). The most common below-ground symptom in maize roots is varying sizes of necrotic

root lesions (Fig. 6.17) (Corbett, 1976; Fortuner, 1976; Bridge and Starr, 2007). Damage to fibrous root systems can result in destruction of the cortical parenchyma and epidermis, which may cause sloughing off of the tissue and severe necrosis (Fig. 6.18). Severe root pruning and proliferation of lateral roots may also occur. *P. zae* causes mechanical breakdown of cells and necrosis of stellar and cortical tissues, resulting in the formation of cavities (McDonald and Nicol, 2005). *P. zae* may cause considerable reductions in root and shoot weight, plant height and chlorophyll content (Patel *et al.*, 2002), whereas *P. brachyurus* causes more necrosis than mechanical damage (Corbett, 1976).

Damage potential and economic importance

Pratylenchus density may increase considerably under continuous maize cropping, resulting in significant yield losses (Reversat and Germani, 1985; Maqbool and Hashmi, 1986; Bridge and Starr, 2007). However, yield loss estimates in



Fig. 6.17. Healthy root (left) and highly necrotic root (right) of maize caused by a severe infestation with the root lesion nematode *Pratylenchus* sp. (Photograph courtesy of D. Coyne.)



Fig. 6.18. Maize root showing typical necrotic lesions along entire root system caused by the lesion nematode *Pratylenchus* sp. (Photograph courtesy of F. Kagoda.)

maize due to *Pratylenchus* spp. are mostly confounded by other factors, although studies using nematicide applications have shown yield increases of 33–128%, 10–54% and 100% in South Africa, the USA and Brazil, respectively (Walters, 1979; Riekert, 1996; McDonald and Nicol, 2005).

Evaluating maize yield losses is complicated further by the complex interactions between lesion nematodes and fungi/bacteria. In South Africa, the effect on maize plant growth was more severe under combined inoculation of *P. zeae*, *P. brachyurus* and *Fusarium moniliforme* than when inoculated with nematodes alone, with the greatest effect during the seedling stage (Jordaan *et al.*, 1987). However, another study reported a reduction in *P. pratensis* population densities when it occurred concomitantly with *F. moniliforme* on maize (Revelo Moran *et al.*, 1993). Inoculation of both *Fusarium* spp. and *P. penetrans* affected maize root health negatively, with more damage observed with *Fusarium verticillioides* than with *Fusarium graminearum* (Silva *et al.*, 2016).

Heterodera

More than ten cyst nematode species have been associated with maize in subtropical and tropical countries, with three (*H. zeae*, *H. avenae* and *P. chalcensis*) considered economically important (Luc, 1986). Swarup *et al.* (1964) first recorded *H. avenae* on maize in subtropical regions of India, although it also occurs in Egypt and Italy (Ibrahim *et al.*, 1986; Bajaj and Kanwar, 2005). The worldwide distribution of *H. avenae* on cereals and information on the biology of this nematode species is discussed in the section under wheat, above.

Heterodera zeae

Distribution

H. zeae was first described from India (Koshy *et al.*, 1970), where it was widely distributed (Sharma and Swarup, 1984). It also occurs in Egypt, Greece, Nepal, Pakistan, Portugal, Thailand and the USA (Ibrahim *et al.*, 1976; Mulvey and Golden, 1983; Aruna and Siddiqui, 1997;

Sharma *et al.*, 2001; Correia and Abrantes, 2005; McDonald and Nicol, 2005; Skantar *et al.*, 2012).

The primary hosts of *H. zeae* are in the Poaceae (barley, millet, oat, wheat, Vetiver grass, *Zea mexicana* (McDonald and Nicol, 2005), although it has been reported from citrus, jute, pear, sweet pepper, tomato (EPPO, 2015) and several weeds (Ringer *et al.*, 1987). Non-graminaceous families, e.g. Malvaceae, Compositae, Cruciferae and Cucurbitaceae, are regarded as non-hosts (Shahina and Maqbool, 1990).

Biology and life cycle

Temperature plays an important role in the biology of *H. zeae* (Ismail *et al.*, 1994; McDonald and Nicol, 2005). The most favourable temperature for optimal emergence (91%) of J2 from cysts is 25°C. At temperatures of 10–15°C, only 10–20% of the J2 emerge. The life cycle from egg to reproducing adult is short (15–18 days) under favourable temperatures (27–39°C), with six to seven generations completed during a growing season (Srivastava and Sethi, 1985; EPPO, 2015).

Generally, the nematode reproduces well in moderately light soils. The addition of clay to soil mixtures resulted in a proportional decline in nematode reproduction levels (Srivastava and Sethi, 1984).

Symptoms of damage

H. zeae-infected plants exhibit poor growth, are stunted and are pale green in colour, with narrow leaves (Koshy and Swarup, 1971; EPPO, 2015). The root systems of infected plants are poorly developed, with a bushy appearance. Maize tassel development may also be noticeably delayed, resulting in smaller cobs with lower grain masses (EPPO, 2015).

Pathotypes

Three host races, based on reproduction and host preference, exist (Bajaj and Gupta, 1994).

Damage potential and economic importance

There is limited data on the economic impact of *H. zeae* on maize, although there seems to be a

direct correlation between plant growth reduction and *H. zae* population density. In Rajasthan, low numbers (six J2/cm³ soil) caused a 29% crop loss (Aruna and Siddiqui, 1997). In Maryland (USA), maize growth and yield were reduced between 13 and 73% due to *H. zae* parasitism, with damage being more severe in coarse-textured soil, and under hot and dry conditions (Krusberg *et al.*, 1997).

Punctodera chalcoensis

P. chalcoensis (syn. *Heterodera punctata*) was recorded on maize roots in Mexico during the 1960s, although distinct morphological differences between the Mexican population and the originally described *H. punctata* were later recognized (cited in McDonald and Nicol, 2005). Furthermore, the Mexican population did not parasitize wheat and grasses, which are common hosts of *H. punctata*. The Mexican population was hence later redescribed as *P. chalcoensis* (Stone *et al.*, 1976).

Distribution

P. chalcoensis (Mexican corn cyst nematode) only occurs in Mexico, where it is considered of extreme importance. Of 300 graminaceous plant species tested, only *Z. mays* and *Z. mexicana* (teosinte) were considered hosts (Stone *et al.*, 1976).

Biology and life cycle

P. chalcoensis completes one generation per year. It survives winter in diapause (Sosa-Moss, 1987) and reproduces well in all soil types, causing severe damage to maize crops in volcanic sandy soils.

Symptoms and economic importance

Maize fields infested with *P. chalcoensis* have patches of stunted and chlorotic plants. Damage can be severe and is dependent on cultivar susceptibility, nematode density and adequate soil moisture levels in the latter part of the growing season. In heavily infested sandy soils, plants are markedly stunted with chlorotic leaves that exhibit pale-coloured stripes. These symptoms can be distinguished from those caused by the virus

disease 'Rayado Fino', where pale, striped lines occur in green leaves.

Maize root systems are generally poorly developed when infected by *P. chalcoensis*; large densities of white females can be observed on roots 2 months after planting. Sosa-Moss and Gonz  les (1973) obtained a reduction of $\pm 60\%$ in maize yield in heavily infested soils.

Other nematodes associated with maize

Numerous other plant parasitic nematodes are associated with maize (McDonald and Nicol, 2005; Bridge and Starr, 2007), yet, in most cases, their impact on maize production has not been determined (Koenning *et al.*, 1999; Bernard *et al.*, 2010). Of limited or local importance are species of *Belonolaimus*, *Criconeimella*, *Ditylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Longidorus*, *Paratrichodorus*/*Nanidorus*, *Quinisulcius*, *Radopholus*, *Rotylenchulus*, *Tylenchorhynchus* and the cyst nematode *Vitattidiera zeaphila* (Landi and Manachini, 2005; McDonald and Nicol, 2005; Hassan *et al.*, 2009; Bernard *et al.*, 2010; Guo and Shi, 2012;   revkov   and Cag  n, 2013).

According to   revkov   and Cag  n (2013), *Bitylenchus dubius* is potentially harmful to maize in temperate climatic zones, while Williams and Beane (1984) consider this species an imminent pest of the crop in the UK. *Longidorus* and *Xiphinema* are also considered pests of maize, causing severe root tip damage on sandy soils, with subsequent yield loss, especially under moisture stress. A negative linear relationship has been demonstrated between yield and *Belonolaimus* spp. population densities (Todd, 1989); *Belonolaimus longicaudatus* can cause severe losses to sweetcorn on sandy soils in Florida (Rhoades, 1977; Bridge and Starr, 2007). Seed-borne *Ditylenchus* juveniles were detected in maize fields (Tenente *et al.*, 2000), and Knuth (2000) reported differential susceptibility of maize cultivars to *D. dipsaci*, with significant effects on seedling development and yield. *Helicotylenchus indicus* also impacts maize, causing significant decreases in chlorophyll content, plant height, dry shoot and root masses with increasing nematode densities (Kumar and Singh, 2007).

Management measures for maize nematodes

CHEMICAL: despite the relatively low economic value of maize, the use of nematicides generally remains the most common control measure, particularly for more commercial systems, or under irrigation or for seed production purposes. Studies provide inconsistent results regarding nematicide use on maize crops (Zondagh and Van Rensburg, 1983; McDonald and Nicol, 2005; Bridge and Star, 2007), but when environmental conditions and all other factors are optimal, nematicide application offers substantial returns to producers. For example, *H. zae* population densities were reduced substantially when a single dose of Cadusafos was applied at planting (Srivastava and Jagan, 2007). Under rain-fed conditions and in subsistence agriculture, the application of nematicides is seldom economically feasible.

The use of maize seed treated with nematicidal products (often in combination with fungicides and insecticides) is a popular and cost-effective nematode management strategy (Van Zyl, 2013). In glasshouse experiments in Brazil, the use of two experimental nematicidal seed treatment products significantly reduced *P. zae* population levels (Confort and Inomoto, 2015). Silva *et al.* (2016) demonstrated that seed treatments containing abamectin and fungicides reduced root infection by *P. penetrans* and provided the healthiest root systems where *Fusarium-Pratylenchus* complexes existed. The potential carry-over benefits of nematicide use on maize – in terms of pest reduction for succeeding crops – should not be ignored (Johnson and Leonard, 1995).

RESISTANCE: introgressing nematode resistance into maize may result in a trade-off with other commercially desirable or preferred traits (Jordaan *et al.*, 1987; Williams *et al.*, 1990). However, breeding without selecting for nematode resistance may result in highly susceptible and intolerant crops, which could be very costly in production systems. Although Jordaan *et al.* (1987) have reported that identification of resistance to migratory nematodes is more difficult than for sedentary endoparasites, numerous maize cultivars are reported to be resistant to various plant parasitic nematode species, such

as *Helicotylenchus pseudorobustus*, *Helicotylenchus sorghi*, *M. incognita*, *M. javanica*, *M. arenaria*, *Nanidorus minor*, *P. brachyurus*, *P. hexincisus*, *P. scribneri*, *P. zae*, *Rotylenchulus reniformis*, *Trichodorus christiei*, *T. vulgaris* and *Tylenchorhynchus zambiensis* (De Brito and Antonio, 1989; Windham and Lawrence, 1992; Windham and Williams, 1994a,b; Gautam and Srivastava, 2005; cited in McDonald and Nicol, 2005; Wilcken *et al.*, 2006; Timper *et al.*, 2007; Inomoto, 2011; Rosa *et al.*, 2012).

Poerba *et al.* (1990) reported general (additive resistance) and specific (single gene dominance) combining resistance to *M. javanica* in diallel crosses between maize inbred lines, with Mp307 the best source of resistance. A later study by Williams and Windham (1992) with inbred line diallel crosses on *M. incognita*, however, showed general combining ability (referring to the average performance of a specific line in hybrid combinations it has been introgressed into) to be a better source of variation. On the other hand, specific combining ability aims to identify those hybrid combinations that perform better or worse than would be expected when compared to the average performance of the lines introgressed into such combinations. The high specific combining ability effects among some parents demonstrated their utility in breeding resistant cultivars that are adapted to a wide range of climatic conditions in Uganda (Kagoda *et al.*, 2011). Such dominant genes will favour pedigree and various other breeding efforts to improve grain yield under nematode pressure.

Open-pollinated maize cultivars were also screened as possible sources of resistance to *M. incognita* in the USA (Aung *et al.*, 1990, 1991), while Ngobeni *et al.* (2011) reported various hybrids and open-pollinated cultivars adapted to South African climatic conditions with resistance to *M. incognita* and *M. javanica*. Kagoda *et al.* (2011, 2015) reported maize hybrids with combined resistance to *P. zae* and *Meloidogyne* spp. These studies showed that nematode tolerant/resistant hybrids had substantially higher yield compared to the checks, with grain yield losses of $\pm 28\%$ recorded for susceptible hybrids, indicating the substantial economic losses experienced as a result of parasitism by prevailing nematode pests. In Nigeria, maize cultivars with resistance to *M. incognita* were also identified (Adegbite, 2011).

Ultimately, the focus of introgressing nematode resistance should be to produce a genotype with acceptable agronomic traits and durable resistance. The use of marker-assisted selection (Young and Mudge, 2002) should be considered, especially where single gene dominance is available. This technology can assist breeders in introgressing the preferred genes from male as well as female parents, ensuring sustainable heritability of resistance traits.

CULTURAL: crop rotation, tillage, planting time, application of organic amendments and sanitation are effective in reducing nematode pests. Several studies on crop rotations involving maize have accentuated the dangers of ineffective crop choices due to the susceptibility of maize to nematode pests (Florini and Loria, 1990; Gallaher *et al.*, 1991; Todd, 1991; Riekert and Henshaw, 1998; Hague *et al.*, 2002). The dangers of targeting a single nematode species are also emphasized, while the implementation of longer sequences of resistant crops before planting a susceptible crop were also demonstrated to be more effective (McDonald and Nicol, 2005). The wide host ranges of plant parasitic nematodes also include several weed species (Salawu and Oyewo, 1999; Ntidi *et al.*, 2012), which should be considered when control strategies are designed. Weeding maize plots reduces populations of *Ditylenchus* spp., *Heterodera* spp. and *Tylenchorhynchus clarus* (Youssef, 1998).

It is possible to use maize as a resistant rotational crop to manage nematodes (Acosta *et al.*, 1991; Rodríguez-Kábana *et al.*, 1991; Davis *et al.*, 2003), particularly where maize is a non-host to cyst nematodes (Noel and Edwards, 1996). While it is sensible to test the host suitability of all crops used in a system to all potentially important nematode pests (Wang *et al.*, 2002), and to use resistant genotypes, rotation alone is rarely sufficient to prevent damage to susceptible succeeding crops. Additional control strategies, such as growing antagonistic crops, e.g. radish or French and African marigold (*Tagetes patula* and *Tagetes erecta*), have been shown to reduce *Pratylenchus* spp. populations in maize-based rotations (Knuth, 2002). Rotating or intercropping maize with bristle oats and oilseed radish also resulted in reduced reproduction rates of *P. brachyurus* in Brazil (Chiamolera *et al.*, 2012). Meanwhile, intercropping maize with

jack bean or velvet bean under glasshouse conditions significantly improved maize growth (up to 34%) and reduced *P. zaeae* population densities (32%). Under field conditions, intercropping maize with jack bean significantly increased maize yields by 22–190% (Arim *et al.*, 2006).

The increasing popularity of conservation tillage and no-till requires a good understanding of the effect of tillage on nematode pest populations, particularly where other nematode management practices are also used and where these tillage regimes are important for reasons other than nematode management (McSorley and Gallaher, 1994). Minton (1986) gave an overview of tillage and the accompanying factors affecting nematodes, while Cabanillas *et al.* (1999) recommended the combination of tillage and crop rotation as part of a nematode management strategy. In some studies, tillage had little effect on nematode densities, while in other studies they were influenced greatly by tillage (Sumner *et al.*, 2002; McDonald and Nicol, 2005). The important influence of environmental factors in these systems and interrelationships cannot be overemphasized (Yeates and Hughes, 1990; Yeates *et al.*, 1993). Repeated tilling of the upper 30 cm soil layer at 30-day intervals during the hot and dry seasons between crops can, however, contribute significantly to reducing nematode pest densities due to desiccation of eggs and infective juvenile stages (Sikora *et al.*, 2005). This practice, however, is not always economical. Another consideration is the loss of soil moisture after tilling and the impact it will have on the current and successive crop. In terms of reduced tillage/soil disturbance as part of conservation agriculture, the application of lime and fenamiphos yielded positive results in a Zimbabwean study where maize yield was correlated negatively with *Pratylenchus* numbers (Madamombe *et al.*, 2017). These two treatments reduced lesion nematode numbers significantly (>10 times) compared to the untreated control, but not that of *Criconema*, *Helicotylenchus*, *Meloidogyne* and *Trichodorus*. Maize yield also increased significantly by 53% for the fenamiphos treatment and 42% for the lime treatment compared to the untreated control.

In Mexico, early sowing dates and adequate fertilizer application reduced damage caused to maize by *P. challoensis* (Sosa-Moss and Gonzalez, 1973; Sosa-Moss, 1987). Krusberg *et al.* (1997)

found no alleviation of damage by *H. zae* to maize by fertilizer amendments, but Ivezić *et al.* (1996) obtained up to 60% reductions in nematode densities dominated by *P. thornei* after applying high levels of potassium. The application of animal litter reduced *Helicotylenchus dihystra* and *Paratrichodorus christiei* in maize fields (Sumner *et al.*, 2002), while the application of compost in large amounts reduced *Criconeoides* spp., *Nanidorus minor* and *Pratylenchus* spp. densities associated with maize (McSorley and Gallaher, 1996). McSorley and Gallaher (1997) ascribed the inconsistent performance of compost against nematode pests to the positive effect of the amendment on crop performance. More consistent effects are observed after the prolonged application of compost, which improves soil organic matter content and water-holding capacity. In Iran, soil nematode population densities, dominated by plant parasitic nematodes, were significantly higher in plots where cow manure was applied (40 t/ha), compared to plots receiving 20 t/ha, and untreated plots (Moradi *et al.*, 2013). The use of green manures of various plant species reduced population densities of *M. incognita*, *H. dihystra*, *T. nudus* and *P. zae* significantly and increased maize plant growth (Thakur, 2014). Under maize monocropping in Nigeria, plant parasitic nematode densities (dominated by *Hoplolaimus* spp., *Pratylenchus* spp. and *Scutellonema* spp.) were reduced by the incorporation of animal manure compared to urea application and intercropping with legumes (Eche *et al.*, 2013). Leroy *et al.* (2007) also found that the addition of vegetable-waste compost and cattle manure to maize monoculture plots reduced the population densities of *Pratylenchus* sp. and other Tylenchidae over a 7-year period. In China, the total nematode density (dominated by *Tylenchorhynchus*, *Pratylenchus*, *Helicotylenchus* and *Rotylenchus*) in soil to which compost was incorporated was generally significantly higher than those in soil treated with chemical fertilizers and the untreated controls (Hu and Qi, 2010). Meanwhile a Nigerian study found that plant parasitic nematode population densities in soil samples were reduced significantly (3.4–80%) by a combined treatment of urea fertilizer and animal manure compared to a treatment of urea only (Mary *et al.*, 2013). In India, *P. sativus* and *Tylenchorhynchus zae* population densities were reduced by 50 and 66%,

respectively, compared to the untreated control, by the addition of individual treatments of 800 kg/ha poultry and pigeon manure (Hassan *et al.*, 2009). The addition of sawdust also reduced *P. sativus* numbers by 45%. Chiamolera *et al.* (2012) recorded lower population densities of *P. brachyurus* in field plots amended with bird litter.

The incorporation of wild sunflower compost into soils infested with *P. brachyurus* significantly reduced densities of this nematode pest in maize, with a resultant increase in yield (Olabiyi, 2013). Zhang *et al.* (2016c) demonstrated that long-term organic fertilization practices in China favoured both plant parasitic and beneficial nematode community build-up in summer maize. However, compared to winter wheat, the biomass carbon content of plant parasites in inorganic fertilized plots (to which cattle manure and wheat straw were also added) decreased by approximately 22 and 52%, respectively, during the maize-growing season. Plant residues and organic fertilizers significantly suppressed *M. incognita* galling and reproduction in maize roots, resulting in a substantial increase in plant growth parameters and yield (Odeyemi *et al.*, 2011). The retention of crop residues substantially reduced *P. thornei* in maize fields in Mexico (Govaerts *et al.*, 2006).

In a pot experiment in Brazil, sewage sludge, vinasse, filter cake, poultry litter and manipueira (liquid extract of cassava roots) all effectively reduced population densities of *P. brachyurus* in maize roots and rhizosphere soil samples (Roldi *et al.*, 2013). In another study, *P. brachyurus* were reduced when sewage sludge was applied to the soil in which maize was planted, but this treatment was not successful in reducing *M. incognita* densities (Fontana *et al.*, 2012).

BOTANICALS: significant reductions in *H. zae* population densities in maize root and in soil samples were recorded after the application of neem (*Azadirachta indica*) leaf powder, with a 46% increase in maize shoot weight (Metha *et al.*, 2015). The use of decomposed orange peel oil resulted in a 51% higher mortality of *P. penetrans* individuals compared to ordinary, fresh orange peel in a Pakistani field experiment. Plants treated with decomposed orange peel oil were also taller and had better yields than those in the untreated control plots (Fabiya *et al.*, 2014). *P. zae* densities were significantly lower

in plants treated with a neem-based product combined with a fungicide (Aly *et al.*, 2009).

BIOLOGICAL: the economical use of biocontrol agents in cereal crops poses a challenge, as the majority of maize is cultivated under harsh, rain-fed conditions. However, there have been successes, such as the growth promotion effects of *Trichoderma* spp. on maize in the presence or absence of *M. arenaria*; this does not necessarily indicate parasitism by the fungus on the nematode, but could be a direct or indirect result of secondary compounds produced by the fungus (Windham *et al.*, 1989). Riekert and Tiedt (1994) provided evidence of *Arthrobotrys dactyloides* trapping of *M. incognita* J2 on maize roots. Several species of nematode-trapping fungi were present in a maize–tomato rotation, although detection frequencies and population densities of such fungi did not differ significantly between organic and conventionally treated plots (Timm *et al.*, 2001). Bourne (2001) obtained a 50% reduction in *M. incognita* numbers after applying *P. chlamydosporia* in rotations with maize and susceptible crops, and Bourne and Kerry (1999) reported significant control of *M. incognita*, *M. javanica* and *M. arenaria* in maize with the application of this fungus.

More than 50% control of *Pratylenchus* spp. was achieved using *P. lilacinum* (Gapasin, 1995), while strains of *Pseudomonas* spp. inhibited invasion of *Meloidogyne* spp. and *Radopholus similis* in maize, tomato and banana roots (Aalten *et al.*, 1998). The use of 4% w/w seed-coat treatments of *P. lilacinum* and *P. chlamydosporia* reduced *H. zaeae* densities in sweetcorn in India by 36 and 25%, respectively, compared to the untreated control, and increased shoot weight by 29 and 24%, respectively (Baheti *et al.*, 2015). Mycorrhizal fungi of the genus *Glomus* reduced *M. chitwoodi* J2 numbers in maize roots (Estanol-Botello *et al.*, 1999). Applications of either *Glomus mosseae* or *Chromolaena odorata* powder were effective in controlling a Nigerian population of *M. incognita* and increasing maize yield (Odeyemi *et al.*, 2013).

Sorghum

Sorghum (*Sorghum bicolor*) is the fifth most important cereal worldwide (<http://www.fao.org/>

[faostat/en/#data/QC](http://www.fao.org/faostat/en/#data/QC)). Sorghum is thought to originate from Africa (Maunder, 2002), where it is primarily produced (see Table 6.1). The crop is also very important in Asia and Central and North America, where production is relatively stable (<http://www.fao.org/faostat/en/#data/QC>). Without irrigation or rainfall after sowing, sorghum yields about 2–4 t/ha with no till, good weed control and agronomic practices (Martin *et al.*, 2015).

Sorghum is an important food and fodder crop of dryland agriculture and is adapted to a wide range of environmental conditions, from semi-arid through temperate to high rainfall areas (Smith and Frederiksen, 2000; Kollo, 2002; Prasad *et al.*, 2008). Sorghum is used to produce various forms of bread in India and Central America, Sudan, Ethiopia and India, or as a porridge in Africa and India. It is also boiled, similar to rice, and used to produce alcoholic and non-alcoholic beverages in some African countries. Furthermore, it can be used for ethanol production in countries such as Brazil (Dahlberg and Frederiksen, 2000; Martin *et al.*, 2015). Sorghum is gluten free and is a useful food source for people who are intolerant to gluten (Prasad and Staggenborg, 2010).

Nematodes of Sorghum

Several nematode species have been associated with sorghum but little information is available on specific nematode problems. Plant parasitic nematodes are responsible for 6.9% losses in sorghum crop production worldwide, with *Pratylenchus* being the most important (Stirling, 2014). Nematode damage to sorghum is most likely in monoculture cropping (Traoré *et al.*, 2012). Globally, species of three genera are listed as important pests of sorghum: root lesion, stunt (*Tylenchorhynchus*) and root knot nematodes (De Waele and McDonald, 2000; Traoré *et al.*, 2012).

Pratylenchus

Pratylenchus spp. are omnipresent and frequently associated with sorghum (De Waele and Jordaan, 1988; Sharma and McDonald, 1990;

De Waele and McDonald, 2000). Conflicting reports exist on the damage potential of lesion nematodes on sorghum, mainly attributed to differences in cultivars, environment and infestation levels (Kollo, 2002). Species frequently associated with sorghum are *P. brachyurus*, *P. coffeae*, *P. crenatus*, *P. goodeyi*, *P. hexincisus*, *P. neglectus*, *P. penetrans*, *P. scribneri*, *P. thornei* and *P. zeae* (McDonald and Nicol, 2005; LaMondia, 2006; Smiley *et al.*, 2014; Stirling, 2014). Infection by lesion nematodes results in necrotic lesions on roots, with heavily infected plants appearing stunted and chlorotic (Chevres-Roman *et al.*, 1971; Bee-Rodriguez and Ayala, 1977; Claflin, 1984; Cuarezma-Teran and Trevathan, 1985; Motalaote *et al.*, 1987). Infected roots have reduced the uptake of nutrients and water from soil. Lesion nematodes also interact with plant pathogens (Bee-Rodriguez and Ayala, 1977; Kollo, 2002). Sorghum is generally a good rotation crop in potato, maize, soybean and lucerne cropping systems (Florini and Loria, 1990; Gallaher *et al.*, 1991; Todd, 1991; Thompson *et al.*, 2008, 2012).

Tylenchorhynchus

Tylenchorhynchus martini, *T. nudus* and *Quinisulcius acutus* (Claflin, 1984; Cuarezma-Teran and Trevathan, 1985) are associated with poor growth of sorghum plants. Sorghum–sudan grass increases *Tylenchorhynchus* spp. densities (Crow *et al.*, 2001), while sorghum is an excellent host to *Tylenchorhynchus ewingi* compared to other small grains, such as ryegrass, oat and wheat (Fraedrich *et al.*, 2012). Both *T. martini* and *T. nudus* increased under sorghum monocultures, while yield increases of 55% were recorded after nematicide treatment where *T. martini* was predominant (Hafez and Claflin, 1982). Similarly, *T. nudus* reduced plant growth by 10 and 56% in fertilized and unfertilized plots, respectively (Smolik, 1977). At least eight more *Tylenchorhynchus* spp. have been reported as pests of sorghum (Kollo, 2002).

Nematode feeding results in poorly developed root systems. Root tips may be short and become swollen, and severely infested fields may result in stunted growth and decline of seedling vigour (Claflin, 1984). Although a 30% reduction

in root fresh weight can be caused by stunt nematodes on sorghum, top growth is less affected (Kollo, 2002). Interactions between plant pathogens and stunt nematodes are reported on sorghum (Sharma and McDonald, 1990).

Meloidogyne

Sorghum is a good host for various *Meloidogyne* spp., such as *M. incognita*, *M. arenaria*, *M. javanica*, *M. naasi* and *M. graminicola* (Sharma and McDonald, 1990; De Waele and McDonald, 2000; Kollo, 2002). *Meloidogyne acronea* has been detected on sorghum in South Africa (Coetzee, 1956) and Malawi (Bridge *et al.*, 1976). Specific races of *M. incognita* and *M. arenaria* are also better adapted to sorghum (Ibrahim *et al.*, 1993; Kollo, 2002), with only race 5 of *M. naasi* recorded parasitizing sorghum (Ediz and Dickerson, 1976). The latter species causes stunting and chlorosis of infected plants. The optimum soil temperature for development is 26°C, and the life cycle is completed in 34 days. Sorghum is seemingly a poor host to *Meloidogyne ethiopia* (Lima *et al.*, 2009).

M. incognita infestation of sorghum results in the production of elongated swellings or discrete knots, and proliferation of roots (Orr and Morey, 1978). Galls produced by *M. naasi* are similar but smaller than *M. incognita* galls (De Waele and McDonald, 2000), whereas *M. acronea* induces extensive root proliferation but inconspicuous root galls (Page, 1985). Screenings of sorghum genotypes against *Meloidogyne* spp. suggest that this crop is generally a poor host to this pest and is therefore a suitable rotation option with more susceptible crops (Fortnum and Currin, 1988). Variable levels of resistance in sorghum cultivars exist, with some reports of high susceptibility and other reports of resistance (Sharma and McDonald, 1990; McSorley and Gallaher, 1992; Ribeiro *et al.*, 2002). Sorghum hybrid cv. Super Dolce 10 is reportedly a poor to non-host of *M. incognita* (Curto *et al.*, 2012; Aminu-Taiwo *et al.*, 2015), while sorghum–sudan grass is a poor host to *M. javanica* (Miyasaka *et al.*, 2016). Forage sorghum is the most frequently used green manure crop, as it improves soil health and reduces *Meloidogyne* spp. (Gard *et al.*, 2014). Sorghum root extracts at

high concentration (20 ppm) and longer exposure (48 h) reduced *M. javanica* activity and increased its mortality (Ali and Pervez, 2009; Lalitkumar *et al.*, 2015). Not many nematode control options exist for sorghum nematodes, due to its low value and the poor growing conditions. Improvement of growing conditions and low-input management practices are therefore recommended (Kollo, 2002).

Other nematodes associated with sorghum

Many other plant parasitic nematode species have been associated with sorghum (De Waele and McDonald, 2000; Kollo, 2002). *Longidorus africanus* and *H. zeae* have been shown to be pathogenic in pot experiments (Lamberti, 1969; Singh *et al.*, 1979). *Mesocriconema ornatum* and *Criconeimoides sphaerocephalus* reproduce well on sorghum (Gallaher *et al.*, 1991; McSorley and Gallaher, 1993), but are not considered economically important (McSorley and Gallaher, 1992). Several weed species are good hosts of *Belonolaimus* spp., thus stringent weed control is required where sorghum is a rotation crop (Todd, 1991). Grain sorghum is also a good rotation crop to reduce pin nematodes *Paratylenchus projectus* (Todd *et al.*, 2014).

Millet

Millets are warm-weather cereals with small grains and comprise the species *Panicum miliare*, *Panicum miliaceum*, *Paspalum scrobiculatum*, *Setaria italica*, *Echinochloa colona*, *Digitaria exilis*, *Eragrostis tef*, *Eleusine coracana* (finger millet) and *Pennisetum glaucum* (pearl millet), collectively known as small millets (Esele, 2002; Hash and Witcombe, 2002). Worldwide, millet crop losses to plant parasitic nematodes are estimated at 11.8% (Stirling, 2014). Millets are an important staple food in India and several countries of Africa, the Near East and South Asia. Despite their importance (see Table 6.1), there is little information on nematode associations with millets, probably because the crop is largely produced in a subsistence context, on marginal soils and

under adverse climatic conditions (Hash and Witcombe, 2002). Millets are grown almost exclusively for animal feed in developed countries (Kollo, 2002).

Pearl millet

Pearl or bulrush millet (*P. glaucum*) is cultivated for grain and fodder in arid regions of Africa, India and Asia, and as pasture in the USA. This crop is highly tolerant to stresses, such as drought, low soil fertility and heat (Kollo, 2002; Sheahan, 2014; Chapuis-Lardy *et al.*, 2015). Several plant parasitic nematode species are associated with pearl millet; it is a host for both *M. incognita* and *M. javanica* (Handa *et al.*, 1971), although genotypes in Brazil are resistant to these two *Meloidogyne* spp. (Ribeiro *et al.*, 2002). In north-western India, *M. incognita* has been reported as a problem when it occurs in combination with *Sclerospora graminicola*. Symptoms of green ear disease caused by the fungus were advanced by about 2 weeks when *Meloidogyne* spp. were present (Vaishnav and Sethi, 1978). Depending on the cultivar, the crop is a poor/non-host for *M. acrona* (Bridge *et al.*, 1976; Page, 1983). *M. arenaria* race 2 populations are suppressed by pearl millet in rotations with soybean, resulting in low gall indices on soybean roots (Kinloch and Dunavin, 1993). Millet in the former USSR is affected by *L. elongatus*, with infected chlorotic plants stunted with shortened, thick and deformed roots and yield reductions of 41% (Semkin, 1975). In glasshouse tests, pearl millet proved to be a favourable host for *T. vulgare* (Upadhyaya and Swarup, 1972).

Several plant parasitic nematode species are considered of variable importance on millet in different countries. *R. reniformis* is a problem for pearl millet in southern India (Seshadri, 1970), whereas an association between a *Fusarium* sp. and *Xiphinema* sp. is reported from Zimbabwe (Sharma and McDonald, 1990). De Waele *et al.* (1998) found 16 plant parasitic nematode species associated with pearl millet in Namibia. Van Biljon and Meyer (2000) found pearl millet to be a good host to *P. delattrei* but not *P. zeae*, whereas pearl millet has good resistance to *P. penetrans* (Belair *et al.*, 2002). Kollo (2002)

provided extensive lists of nematode species and their reproductive potentials on pearl millet. Variable levels of resistance in pearl millet breeding material against *M. incognita* and *M. arenaria* exist (Timper *et al.*, 2002). Dallemole-Giaretta *et al.* (2011) found that pearl millet reduced galls and eggs of *M. javanica* on tomato by more than 90% when used as summer cover crop.

Finger millet

The only nematodes of importance on finger millet (African millet, *E. coracana*) are the cyst nematodes *Heterodera gambiensis* and *Heterodera delvii*, both recorded from southern India and Gambia (Bridge *et al.*, 1978), as well as *R. reniformis* (Shadri, 1970; Krishna Prasad and Krishnappa, 1982). However, finger millet was also reported to be immune to *R. reniformis* (Gardiano *et al.*, 2012) and a non-host (Das and Gaur, 2012) and host to *H. zeae* (Srivastava and Jaiswal, 2011).

Conclusions

Several genera and species of nematodes are of economic importance to small grain cereals. More is known about some nematodes, e.g. *H. avenae*, *Meloidogyne* and *Pratylenchus* spp., than others, with respect to both their biology and control measures. Management of these three nematodes is primarily in the form of host resistance, although others (such as *A. tritici*), are controlled relatively easily with the adoption of seed hygiene. Although maize nematode research has increased significantly over the past decade, barley, sorghum and millets have not received the same attention; in some regions, nematodes may cause significant economic damage to these crops. Previously, cereals were considered poor hosts of *Meloidogyne* spp., but it has become apparent that this genus is very important on cereals, particularly maize.

While management of nematode pests on cereals in developed countries generally relies on synthetic nematicides, the use of alternative management options has and is increasing. Crop rotation is aimed mostly at the inclusion of resistant cultivars and/or crops antagonistic to the

target nematode species. Compared to the number of nematode-resistant cereal cultivars available a few decades ago, substantial inroads have been made, with more now identified and available. The cost of chemicals may be prohibitive and, in many cases, environmentally unacceptable to the average dryland and subsistence cereal producer, but it is unrealistic to expect and believe that their use will be totally terminated. Reducing yield losses caused by nematodes will increasingly require the responsible use of available synthetically derived nematicides, with a greater focus on environmentally friendly and tailor-made management strategies. Furthermore, a greater understanding of basic nematode biology, their interaction with other microbes and the application of appropriate control measures should be obtained.

It is therefore inevitable that breeding for resistance, and perhaps tolerance (although not preferred), is a major strategy for long-term and environmentally sound control of these pests as part of an integrated management approach. Although an increasing number of studies have identified sources of resistance, their use is dependent on commercialization and demand for these genotypes in tropical and subtropical regions. To capitalize on this information, it is necessary to combine research efforts, particularly for some of the more complex nematode pests with race and pathotype differences. The application of biological control agents to reduce nematode pests effectively under field conditions is another strategy that is increasingly being researched and will undoubtedly play a larger role in future as part of integrated pest management systems.

The need for global collaborative research programmes is great. A good example is that of the International Maize and Wheat Improvement Center (CIMMYT), particularly by the soil borne pathogens programme (Turkey). This collaboration between a wide range of international and local institutes/universities focuses on studying cereal nematodes, including root lesion and cyst nematodes, as well as dryland crown rot caused by *Fusarium* spp. The programme also launched the international cereal cyst nematodes initiative workshop in 2009 (now known as the International Cereal Nematodes Symposium) to address nematode problems of small grain cereals (Dababat *et al.*,

2015a). Furthermore, the adoption of molecular tools to assist in both pathogen identification and plant breeding will become an integral part of future research developments and control of important nematode pests. There will be new challenges for nematologists studying cereal nematodes, particularly concerning the introduction of more genetically modified crops, organic crop production, a renewed focus on

conservation practices and changes in global rainfall and temperatures.

Countries with more developed research programmes should assist developing countries with research facilities and manpower. The growing human population ultimately emphasizes the need for sustainable food production, which is in many cases crippled by plant parasitic nematodes.

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7 Nematode Parasites of Potato and Sweet Potato*

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Root and tuber crops are among the most important food commodities. Potatoes, *Solanum tuberosum*, rank third in human consumption after rice and wheat (Devaux *et al.*, 2014). They are produced mainly in the temperate zones but also in many subtropical and tropical countries. Approximately half of the world potato production takes place in Asia and roughly one-third of potatoes are produced in Europe. In 2014, 7% of the world potato output (tons) was produced in Africa, 6.5% in North America, 4% in South America, while production in Central America and Oceania was 0.6% and 0.4%, respectively (FAOSTAT, 2016). No other single crop except maize is grown in more countries. The amount of land in potato production has rapidly overtaken all other food crops in developing countries. It is a fundamental crop for food security for millions of people across South America, Africa and Asia. Presently, more than half of the global potato production is grown in developing countries (Devaux *et al.*, 2014). Potatoes are not only one of the major staple foods worldwide but they are also becoming increasingly important for the processing industry, as global consumption of potato as food is shifting from fresh potatoes to added-value, processed food products.

Sweet potato, *Ipomoea batatas*, a native of tropical America, is more widely grown in

developing countries than any other root crop as a food and feed crop. It is grown in tropical, subtropical and warmer temperate zones. Sweet potato is the second most important root and tuber crop. At present, roughly 80% of sweet potato production is in Asia, 15% in Africa and 3% in the Americas, while production in Oceania and Europe is 0.7% and 0.1%, respectively (FAOSTAT, 2016). Sweet potato is considered an important staple food in most parts of the world, due to its high energy, fibre (Mei *et al.*, 2010) and protein content (Bovell-Benjamin, 2007). Due to its relatively low cost of production, it is affordable to households with limited income. Cultivation of dual-purpose sweet potato in mixed crop–livestock systems in low-income countries has been shown to result in improved livelihoods of farmers (Claessens *et al.*, 2008).

This chapter covers two of these important crops, the potato (*Solanum tuberosum*) and the sweet potato (*Ipomoea batatas*). All other root and tuber crops are discussed in Chapter 8 of this volume.

Potato

S. tuberosum, originating from the Andean highlands of South America, is a major food crop in many countries. According to Hijmans

*A revision of the chapter by M.I. Scurrah, B. Niere and J. Bridge in the second edition.

and Spooner (2001), *Solanum* species occur in America from 38°N to 41°S, and most wild potatoes in South America are found at altitudes between 2000 and 4000 m above sea level. The highest numbers of wild *Solanum* species occur in northern Argentina, central Bolivia, central Ecuador, central Mexico and south and north-central Peru.

Potato is the only tuber crop produced in any significant amount in developed countries. In recent years, potato production has spread gradually out of its traditionally main production areas in temperate regions into hotter and, generally, drier areas in subtropical and tropical countries. Temperature, however, is a main limiting factor for potato production. Potatoes will grow between roughly 10°C and 30°C, with an optimum around 18–20°C.

Nematodes of Potato

Nematodes recognized as major parasites of potato are species of: *Globodera*, *Meloidogyne*, *Ditylenchus*, *Pratylenchus* and *Nacobbus aberrans*. Other nematodes, such as *Belonolaimus longicaudatus*, *Radopholus similis* and *Rotylenchulus reniformis*, have been reported to be associated with potato but are of minor importance (Winslow, 1978a,b; Jensen *et al.*, 1979).

Globodera

The most important parasitic nematodes of potato are the potato cyst nematodes (PCN), *Globodera pallida* and *Globodera rostochiensis* (Evans and Haydock, 1990; Marks and Brodie, 1998).

Potato cyst nematodes are highly specialized parasites of plants with a very narrow host range. Like all cyst nematodes, juveniles invading roots establish a feeding site in susceptible host plants and become sedentary. Nematode eggs within the cysts will persist for many years. Because of their long-term survival in soil once introduced into a field, and because their eradication, containment and control is extremely difficult, PCNs are considered quarantine organisms in more than 100 countries.

To prevent their introduction and spread, they have become the most widely regulated plant parasitic nematodes in the world (Hockland *et al.*, 2013).

Species

Apart from *G. pallida* and *G. rostochiensis*, several other species within the genus *Globodera* have been described parasitizing potato, as summarized by Subbotin *et al.* (2010). *Globodera ellingtonae* was recently described from Oregon (USA) associated with potato (Handoo *et al.*, 2012). This species is suspected to occur in Idaho (USA), Argentina and Chile (Skantar *et al.*, 2011; Lax *et al.*, 2014). It shares characteristics of *G. pallida* and *G. rostochiensis*, but it appears to be more closely related to *G. rostochiensis* (Subbotin *et al.*, 2010). However, the pathogenicity of *G. ellingtonae* to potato is not clear at present (Zasada *et al.*, 2013). *Globodera leptonepia* is considered a rare species (Subbotin *et al.*, 2010). From South African potato fields, *Globodera capensis* has been described, but potato appears not to be a host to this nematode (Knoetze *et al.*, 2013).

Origin and distribution

Potato cyst nematodes originated from South America, where they have evolved together with their principal host plant, the potato. *G. pallida* is mainly present in northern South America, and populations from the area around and south of Lake Titicaca are mainly *G. rostochiensis* (Evans *et al.*, 1975). They were probably introduced into Europe in the 19th century on South American potatoes imported for breeding purposes (Turner and Evans, 1998). Evidence for only a few introductions of *G. pallida* has been produced (Plantard *et al.*, 2008). Only a fraction of the variability found in South America was introduced into Europe, from where the nematodes have been spread further (Evans and Stone, 1977). Europe is hence considered a secondary distribution centre, which is supported by the fact that *G. pallida* populations present in Europe, Asia, North America and Oceania are genetically similar and can be distinguished from populations present in South America (Madani *et al.*, 2010; Subbotin *et al.*, 2011).

Direct introductions of PCNs from South America to other parts of the world may also have occurred, such as the introduction of a Japanese population of PCN (Inagaki and Kegasawa, 1973). Although they are distributed mainly in temperate regions of the world, PCNs are also found in cooler areas of subtropical and tropical regions (Figs 7.1. and 7.2).

Biology

Second-stage juveniles inside eggs remain viable in cysts for over 20 years in soils, even under severe environmental stress (Turner and Evans, 1998). After emergence, juveniles will invade roots, induce a feeding site (syncytium)

and become sedentary. The mechanisms involved in establishment have been reviewed by Gheysen and Mitchum (2011). Male nematodes leave the roots after the final moult, whereas female nematodes remain sedentary (Figs 7.3 and 7.4).

The posterior end of the mature female ruptures the root tissue, but remains attached to the inside of the root. The fertilized females become globose and go through a sequence of colour changes, from white to yellow depending on species (*G. pallida* remains white, whereas *G. rostochiensis* turns yellow), before they finally die and turn into brown cysts (Fig. 7.5). Cysts contain as many as 500 nematode eggs (Evans and Stone, 1977). The size of a cyst is approximately 0.5 mm in diameter (Fig. 7.6).

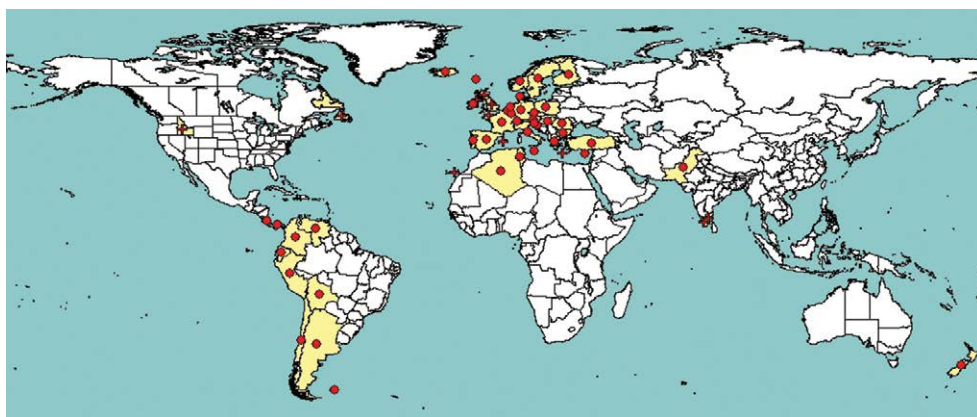


Fig. 7.1. Distribution map of *Globodera pallida*. (From EPPO, 2014.)

Note: red dots = present national records; red + = present subnational records.

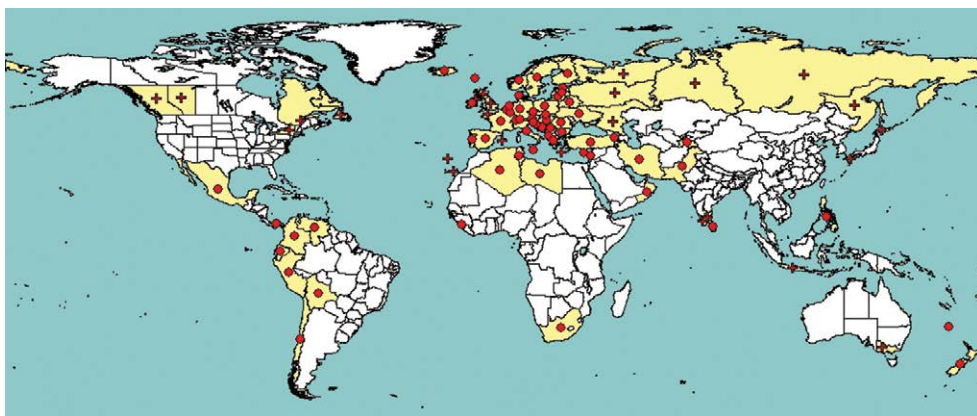


Fig. 7.2. Distribution map of *Globodera rostochiensis*. (From EPPO, 2014.)

Note: red dots = present national records; red + = present subnational records.



Fig. 7.3. Females of *Globodera pallida* developing on potato, *Solanum tuberosum*, roots. (Photograph courtesy of J. Mwangi, JKI, Germany.)



Fig. 7.4. Acid fuchsin-stained female of *Globodera pallida* on the root of potato, *Solanum tuberosum*. (Photograph courtesy of J. Mwangi, JKI, Germany.)

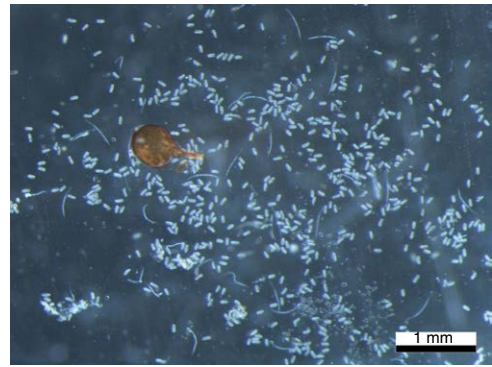


Fig. 7.6. Crushed cyst of *Globodera pallida* with eggs and juveniles. (Photograph courtesy of B. Niere, JKI, Germany.)

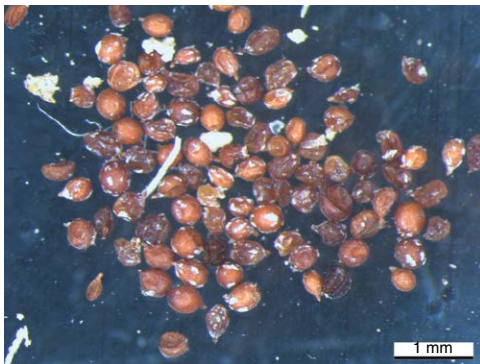


Fig. 7.5. Cysts of *Globodera rostochiensis* after extraction from a soil sample. (Photograph courtesy of B. Niere, JKI, Germany.)

Langeslag *et al.* (1982) observed a base temperature for development of *G. rostochiensis* and

G. pallida of around 6°C and 4°C, respectively. Kaczmarek and co-workers (2014) found that hatch of juveniles was greatest between 15 and 27°C for *G. rostochiensis* and between 13 and 25°C for *G. pallida*. The number of degree days (DD) for the completion of the life cycle was determined by Ebrahimi *et al.* (2014) at 398 DD6 (base temperature of 6°C) for *G. rostochiensis* and at 450 DD4 (base temperature of 4°C) for *G. pallida*. In general, PCNs in temperate regions complete one generation during a growing season. Under favourable conditions, however, a second generation of *G. rostochiensis* (Greco *et al.*, 1988) or *G. pallida* may be observed (Blok *et al.*, 2011).

A large portion of eggs will hatch only if they are stimulated by potato root exudates (Perry *et al.*, 2013). Hatch might also occur without the presence of host plants, and this spontaneous hatch might contribute to the natural

decline of PCN populations. The rate of decline depends on many factors, with soil moisture, temperature and the presence of non-host crops exerting an influence on egg survival inside the cysts. Decline rates have been estimated at between 20 and 30% annually in Europe, whereas in New Zealand 30–70% decline of PCNs, depending on nematode species and soil type, has been observed (Marks and Brodie, 1998). In dry areas of the Andes, the decline is less, and hence longer rotations are needed to reduce populations to below damage thresholds.

Virulence, pathotypes and virulence groups

Virulence is defined as the ability of a nematode to overcome resistance genes (Trudgill, 1991). Virulent PCN populations able to multiply on resistant plants were observed soon after resistance sources were identified. Schemes differentiating populations according to their virulence were established by Canto Saenz and de Scurrah (1977) and Kort *et al.* (1977). Both schemes use the term 'race' or 'pathotype' to designate populations based on their ability to reproduce on differential potato clones. Kort *et al.* (1977) described in the European scheme five pathotypes within *G. rostochiensis* (Ro1 to Ro5) and three pathotypes within *G. pallida* (Pa1 to Pa3). A greater diversity in virulence was described in the South American than in the European scheme (Canto Saenz and de Scurrah, 1977). A continuum in virulence within the European pathotypes Pa2 and Pa3 was reported by Phillips and Trudgill (1998) and that the pathotypes could not be distinguished reliably (Nijboer and Parlevliet, 1990). It was suggested that the term 'virulence group' be used for these populations instead of pathotypes (Anon., 1985). The pathotype schemes have been reassessed by several authors (Stone, 1985; Trudgill, 1985; Nijboer and Parlevliet, 1990), and a summary is given by Fleming and Powers (1998).

Because Europe is considered the secondary centre for PCN distribution, virulence found in European PCN populations has spread through trade in potato to other parts of the world. The introduction of 'new' populations from South America into Europe or other parts of the world could therefore have great implications for potato production systems based on European cultivars with resistance to PCNs (Grenier *et al.*,

2010; Hockland *et al.*, 2012). Phillips and Trudgill (1998) showed that populations from South America were highly virulent on European potato cultivars.

Besides the fact that highly virulent populations are present in South America, the virulence spectrum in Europe, particularly in the virulence group Pa2 and Pa3 of *G. pallida*, has not been fully portrayed. It is also clear that changes in nematode population structure that threaten potato production systems will continue to occur. The predominance of *G. pallida* in the UK has been attributed to the widespread cultivation of cultivars with Ro1-resistance acting against *G. rostochiensis* (Minnis *et al.*, 2002). Selection for virulence within the Pa2 and Pa3 virulence groups has been proven experimentally (Turner *et al.*, 1983; Pastrok *et al.*, 1995; Schouten and Beniers, 1997; Fournet *et al.*, 2013). Although it might take repeated cropping of resistant potato for such a selection to occur, a *G. pallida* population virulent on Pa3-resistant potato cultivars was detected in an agricultural field (Niery *et al.*, 2014). This emphasizes the need to minimize selection pressure by growing potato in long rotations with appropriate volunteer potato control. Despite the fact that the selection of new virulent phenotypes will continue to occur, the cultivation of resistant potato cultivars remains the most environmentally friendly and economically sustainable control measure on infested land.

Symptoms of damage

There are no specific above-ground symptoms associated with PCN infections. However, root injury causes stress and reduces the uptake of water and nutrients, which in turn may cause stunting, yellowing and wilting of the foliage, especially under drought conditions. Shoot growth is reduced by PCNs, and early senescence is often associated with nematode infection (Trudgill and Cotes, 1983). In many cases, tuber weight reductions may occur without any visual above-ground symptoms (Schomaker and Been, 2013). At high nematode population densities, the potato canopy will not achieve full ground cover, whereas at lower densities ground cover will only be achieved at a later stage in canopy development. The random distribution of PCN infestation foci in fields once introduced usually leads to scattered patches of poor growth. It should be noted that several other factors may

lead to poor growth of potato, and therefore this symptom is not specific to PCN infestations. Plant damage is influenced strongly by PCN density in the soil and by the tolerance level of the potato cultivar; that is, the ability of the plant to withstand or recover from nematode damage (Trudgill, 1991).

Spread

The infective juveniles of PCNs only move a distance of about 1 m in the soil. PCNs are usually disseminated passively by the movement of infested soil within a field. As a precautionary measure, the movement of soil on seed potatoes, on machinery, or from a processing plant should be restricted. Seed potatoes should be produced in PCN-free soil, and therefore it is essential that fields for seed potato production be kept free from these nematodes. Substantial movement of soil with farm machinery and on potato crop residues has been demonstrated (Auerswald *et al.*, 2006; Ruysschaert *et al.*, 2006). Therefore, plant residue, machinery, containers, bags and all equipment that has been used on PCN-infested fields should be cleaned. Contaminated soil from processing plants should not be returned to agricultural land without treatment. Composting and heat treatment are effective against PCNs. Irrigation water can also disseminate the nematodes and needs to be regulated on individual and across adjacent areas (Jones, 1970).

Clean planting material along with clean equipment is the best way to prevent the introduction and spread of PCNs. Once potato cyst nematodes are introduced into a field, it is extremely difficult to eradicate them. Successful eradication has only been documented for a small area of 15 ha following a 24-year eradication programme in Western Australia (IPPC, 2010). The prospect for eradication should therefore be evaluated carefully and only considered under special circumstances.

Spread to new areas may not be fully prevented, due to the international movement of seed potatoes and problems associated with the detection of low PCN contamination. It is generally estimated that at least three host crops are required for PCNs to develop to detectable levels, and this is dependent on a number of factors, including crop rotation, host-crop resistance and the genetic constitution and size of the introduced population. The absence of specific

symptoms caused by PCNs further complicates early detection. Fields unknowingly infested may aid PCN spread to uninfested fields or production areas.

New areas in tropical or subtropical regions continue to be found infested (Indarti *et al.*, 2004; Hlaoua *et al.*, 2008). In 2014, *G. rostochiensis* was detected in Kenya (Mwangi *et al.*, 2015), even though this species was considered absent 20 years earlier (Njuguna and Bridge, 1998).

Environmental factors

The conditions that favour successful potato production are also favourable for nematode multiplication and survival. They flourish in cool soil temperatures. High soil temperatures for prolonged periods will limit development and reproduction (Jones, 1970). Soil moisture of field capacity will enhance juvenile movement, while soil nutritional status has no effect on PCNs, other than that caused by crop performance. PCNs tolerate the same soil pH as the potato plant (Jones, 1970).

Other hosts

PCNs are host specific and have a limited host range. Aubergine, tomatoes and a few solanaceous weeds are known to harbour the nematodes, but are not considered to be efficient hosts (Evans and Stone, 1977).

Disease complexes

Potato cyst nematodes provide entry sites for fungi and bacteria. Greater yield losses were observed when *Verticillium dahliae* was present (Storey and Evans, 1987). Interactions have been reported between *G. pallida* and *Pseudomonas solanacearum* (Jatala *et al.*, 1976), *V. dahliae* (Harrison, 1971; Franco and Bendezu, 1985) and *Rhizoctonia solani* (Back *et al.*, 2006).

Economic importance

PCNs are the most important nematode pests of potato. Losses are influenced by several factors, such as potato cultivars, nematode species, virulence type and population density, as well as soil type and biological and environmental factors (Van Riel and Mulder, 1998). Overall losses of 9% of potato production due to PCNs are estimated by Turner and Subbotin (2013). However,

when PCNs are left uncontrolled, total loss of the crop may occur. Yield losses of as high as 80% in some potato-growing areas have been reported from Bolivia (Franco *et al.*, 1998). These high yield losses may have also been caused by *N. aberrans*, another important nematode parasite also present in Bolivia (Lax *et al.*, 2008).

Despite direct yield losses, there will be increased costs for the production of potatoes when PCNs are present, including increased amounts of fertilizers or the application of nematicides. Restrictions on land use because of phytosanitary measures following the detection of infestations may also contribute to the economic importance of PCNs. In intensified potato-production systems, economic consequences are likely to be high, because of shortened rotations or multiple cropping of potato, which will eventually lead to high PCN populations if no control measures are applied.

Management measures

Crop rotation is an effective control measure (Van Riel and Mulder, 1998). For crop rotation to be effective, all hosts including volunteer potatoes should be absent. All non-host crops reduce PCN population densities. Sikora (1984) developed a number of unique rotations for the control of PCNs in the upland tropical growing areas of the Philippines, where multiple cropping of potato was possible. Standard rotations of 6–8 years in Europe are considered sufficient to control PCNs. However, such long rotations are often not feasible in intensive production systems. Therefore, other control measures need to be applied.

Cultivation of resistant cultivars is effective in controlling PCNs. Resistant cultivars can reduce nematode populations in the field by 60–90% (Van Riel and Mulder, 1998). Potato cyst nematode hatches and invades the roots of resistant potato cultivars, but does not complete its life cycle. Pathotype schemes, although considered imperfect, are still in use for the classification of potato cultivars resistant to PCNs (EPPO, 2006). In order to select potato cultivars with appropriate resistance, determination of the virulence of the detected population is necessary. However, this remains difficult, because quick molecular methods for virulence determination are currently not yet available and

therefore a bioassay is required (EPPO, 2013). Bioassay tests can take up to 2 years, especially when prior multiplication of the nematode population is required. Cysts detected during sampling also may not represent the full spectrum of virulence found in the field. Species determination alone, however, helps the selection of suitable test cultivars.

The use of resistance has an immediate selective effect on the population, as outlined above under virulence. Therefore, a management system designed to delay selection of virulent populations is necessary in order to prolong the effectiveness of resistant cultivars. Selection for virulence does not come at a fitness cost for the nematode (Beniers *et al.*, 1995; Fournet *et al.*, 2016). This means the reproduction potential of virulent nematodes is comparable to that of avirulent ones. Therefore, rotations should not include susceptible cultivars, as these will lead to increased population densities. Rotations with long intervals between the cultivation of resistant potatoes can suppress population densities successfully. Cultivars with different resistance genes or alternating cultivars with different resistance genes should also be part of a management system. This recommendation, however, is currently of limited value, as, in most commercial cultivars, resistance to *G. pallida* Pa2 and Pa3, the most difficult group of PCNs to control, is based on polygenic resistance derived from few resistance sources (Gebhardt and Valkonen, 2001). The search for new resistance sources and development of new resistant cultivars is expected to enable the use of these rotations in the future.

Trap crops that elicit hatching and prevent the reproduction of cyst nematodes are an important component in control programmes for cyst nematodes (Scholte, 2000). Franco *et al.* (1999) considered certain cultivars of barley and oca (*Oxalis tuberosa*) useful in inducing hatch of PCN. *Sinapis alba* or *Raphanus sativus*, which are used successfully to control the sugarbeet cyst nematode, *Heterodera schachtii*, however, do not induce hatch of PCNs (Valdes *et al.*, 2011). *Sinapis sisymbriifolium* has also been proposed for use as a trap crop for PCNs, since it can reduce nematode populations by 70–80% (Scholte and Vos, 2000). However, caution needs to be taken when introducing plant species non-native to an area. *S. sisymbriifolium* originates from South America, and has

become a noxious weed of agricultural land in South Africa (Hill and Hulley, 2000). Invasive alien plants are difficult to control, and attempts at biological control of this species have been unsuccessful (King *et al.*, 2011). Although resistant potato clones have been proposed as trap crops by Turner *et al.* (2006), their impact may be similar to growing resistant potato as a main crop.

Brassicaceae, in particular *Brassica juncea*, may be used for biofumigation, even though they do not act as trap crops when used in this manner. Biofumigation crops release glucosinolates after incorporation that can have a direct nematocidal effect on PCNs (Ngala *et al.*, 2015).

Biological control of PCNs has been achieved following application of the fungus *Pochonia chlamydosporia* under UK field conditions (Tobin *et al.*, 2008). Other studies using biocontrol agents such as the fungi *Purpureocillium lilacinum* and *Trichoderma viride* or the bacteria *Pseudomonas fluorescens* and *Rhizobium etli* for PCNs have shown potential under controlled conditions (Hasky-Günther *et al.*, 1998; Devrajan *et al.*, 2011; Umamaheswari *et al.*, 2012; Trifonova *et al.*, 2014). Biological control has also been reported following the application of arbuscular mycorrhizal fungi (Deliopoulos *et al.*, 2008). The full potential of these options needs further evaluation and optimization under field conditions.

Fumigant and non-fumigant nematicides will reduce early infection processes and cause yield improvement in most cases. A reduction in nematode populations can occur when pre-plant densities are low (Evans and Haydock, 2000; Trudgill *et al.*, 2003). At higher initial densities, nematicides may not prevent yield losses nor reduce nematode multiplication (Trudgill *et al.*, 2003). Depending on the nematicide mode of action and degree of biodegradation, nematode populations can reach the same pre-plant densities, or in some cases even increase in size after harvest (see Chapter 23, this volume). Nematicides that are registered in some countries for nematode management are listed in Chapter 23, this volume. New active compounds for the control of PCNs are currently under investigation (Norshie *et al.*, 2016). Although consumer and environmental concerns are making farmers look more closely at non-chemical alternatives, chemical control is still a key management tool in many countries where PCNs are a problem.

In all situations where PCNs are a problem, an integrated management programme is the preferred and most economic means of keeping populations below the damage threshold, reducing dissemination, as well as preventing the emergence of new virulent PCN populations.

Detection and diagnosis

Early detection and proper identification are the first steps to preventing PCN spread. Systematic surveys for the detection of potato cyst nematodes are an important instrument to establish the presence of PCN species or pathotypes/virulence groups in an area.

One way to determine if symptoms of poor growth are caused by *Globodera* is to uproot plants carefully, with as many roots as possible for examination. It is labour-intensive and is considered a very sensitive means of detecting low population densities (Wood *et al.*, 1983). However, as females are only evident on roots for a short period, the examination of roots by lifting plants may not be a reliable method of detection for many times of the year. Soil sampling is a standard procedure in many potato-producing areas as a pre-planting requirement for the production of seed potatoes (Anon., 2007). The probability of detecting PCNs increases with the amount of soil taken and analysed (Been and Schomaker, 2000). Soil sampling should be extensive enough to detect PCNs before visible symptoms are evident. It is important to remember that it takes several years from the time of introduction until the PCNs become established and reach the detection level (Trudgill *et al.*, 2003).

Several protocols for the identification of *Globodera* species exist. A detailed summary on the identification of PCNs and related species is given by Subbotin *et al.* (2010). The European and Mediterranean Plant Protection Organization (EPPO) provides a diagnostic protocol that assists in the morphological and molecular identification of PCNs (EPPO, 2013).

Meloidogyne

Root knot nematodes, *Meloidogyne* species, are considered the most economically important group of parasitic nematodes attacking plants. They are cosmopolitan and attack almost all

major crops and many weed species. The most important species, *Meloidogyne incognita*, has been considered the single most damaging crop pest in the world (Trudgill and Blok, 2001). Approximately 100 species have been described (Karssen *et al.*, 2013), but only a few species have been associated with potato, and then mostly in the temperate zone: *Meloidogyne hapla*, *Meloidogyne chitwoodi*, *Meloidogyne fallax* and *Meloidogyne minor*, although the latter is most likely more a pest of turf (Wesemael *et al.*, 2014). Three species of *Meloidogyne* are considered important on potato in the subtropics and tropics, with *M. incognita* the most widely distributed, followed by *Meloidogyne javanica* and *Meloidogyne arenaria*.

Symptoms of damage

There are no specific above-ground symptoms. Infected plants exhibit stunting and yellowing, and tend to wilt under moisture stress. Infected roots and tubers will have galls or knots of various sizes and shapes (Fig. 7.7).

The number and size of root galls is dependent on nematode density and the nematode species. Galls produced by *M. hapla* and *M. chitwoodi* are usually smaller than those caused by other species. *M. incognita* and especially *Meloidogyne enterolobii* produce large and distinctive root galls.

Under favourable environmental conditions, tubers can become infected (Jatala, 1975). Infected tubers may show warty or pimple-like swellings on the surface. The penetration depth of tubers varies but, depending on the tuber size, nematode females are usually found 1–2 mm

below the skin, feeding on vascular tissue (Jatala, 1975). All species produce necrotic spots in the region between the tuber surface and the vascular ring as a reaction to the deposition of eggs and the gelatinous matrix.

Biology

The biology and life cycle of root knot species on potatoes follow the general patterns described for this genus (Chapter 2, this volume). Although both roots and tubers are infected, the first generation occurs mainly on the root system, with succeeding generations penetrating the tubers (Pinkerton *et al.*, 1991). Several generations are completed on susceptible hosts under favourable environmental conditions, with *M. incognita* producing up to 12 generations in a growing season (Santos, 2001).

Since *Meloidogyne* species attack a large number of plant species, their population can be maintained between potato crops on weeds and volunteer potatoes. However, in the absence of a suitable host, their populations are reduced quickly, because of the lack of a survival stage like cyst nematodes.

Races

There are several populations or host races of *Meloidogyne* species (see Chapter 10, this volume) that infect potatoes. Difficulties in assigning populations to host races and in assessing the full variation of virulence within a population make the practicality of the host race concept questionable (Moens *et al.*, 2009).

Spread

Root knot infected tubers (seed potatoes) and transplants of other hosts of the nematode, as well as the movement of infested soil by farm machinery, and irrigation water are the main pathways for the entry and spread of *Meloidogyne* species.

Environmental factors and other hosts

M. incognita, *M. javanica* and *M. arenaria* develop better at higher temperatures and cannot withstand cool temperatures. Hence, they are of great economic importance in the tropics, subtropics



Fig. 7.7. *Meloidogyne chitwoodi* galls and damage on tubers of potato, *Solanum tuberosum*. (Photograph courtesy of B. Niere, JKI, Germany.)

and warm temperate regions of the world. *M. hapla*, *M. chitwoodi*, *M. fallax* and *M. minor*, on the other hand, are cool-temperature nematodes and have an optimum temperature of 20°C (Taylor and Sasser, 1978). *M. chitwoodi* is reported to be mainly a temperate root knot nematode species, but is also present in Turkey (Devran *et al.*, 2009; Özarslandan *et al.*, 2009). Onkendi and Moleleki (2013) found *M. incognita*, *M. arenaria*, *M. javanica*, *M. hapla*, *M. chitwoodi* and *M. enterolobii* associated with potato in South Africa. Root knot nematode species therefore may be expected to occur wherever temperature conditions are favourable for potato production. *Meloidogyne* species have a wide host range and attack many agriculturally important crops and weeds. Most of the tuber-bearing *Solanum* species are susceptible to root knot.

Disease complexes

Species of *Meloidogyne* often interact with other pathogens in the development of disease complexes. Perhaps the most important interaction of these nematodes on potatoes is their association with *Ralstonia solanacearum* (Jatala *et al.*, 1975; Siddiqui *et al.*, 2014). Resistance in potatoes to bacterial wilt is broken in the presence of *M. incognita* (Jatala *et al.*, 1975; Jatala and Martin, 1977). Other interactions include their association with *Verticillium* spp. and *R. solani*.

Economic importance

Although losses vary depending on the cultivar and environmental conditions, losses can reach 25% or more (Mai *et al.*, 1981). Loss consists of direct damage to the plant, as well as reduction in tuber quality. Infected tubers are economically undesirable and can serve as an inoculum source (Jatala, 1975). A survey in Indonesia found that 97% of the fields destined for potato cultivation were heavily infected with root knot nematode (Suri and Jayasinghe, 2002). Were *et al.* (2013) found root knot nematodes in 38% of the samples of a survey in Kenya.

The cosmopolitan presence of root knot nematodes worldwide and the presence of *M. hapla*, *M. fallax* and *M. chitwoodi* infestations in large commercial potato-growing areas in north-western USA and in Europe, as well as the expansion of potato cultivation into warmer areas,

has increased the importance of root knot management to reduce damage and spread.

Management measures

The use of resistant cultivars and rotation with non-host crops are probably the most practical and economical means for controlling *Meloidogyne*. Potato genotypes with resistance to *M. chitwoodi* have been identified (Norshie *et al.*, 2011; Teklu *et al.*, 2016), but these genotypes have not been evaluated under tropical conditions. Crop rotation with crops resistant to *M. incognita*, such as tomato and sweet potato, will keep population densities low. The application of the bio-control fungus *P. chlamydosporia* in combination with inducers of plant defence (benzothiadiazole) has been suggested as a control strategy (Vieira dos Santos *et al.*, 2014).

Diagnosis

Sampling and extraction procedures are presented in Chapter 4 of this volume. Bioassays using pots filled with field soil in which susceptible tomatoes are planted and subsequently checked for root galling after 4 weeks has proved a reliable method to determine the presence of *Meloidogyne*.

Nacobbus aberrans

The false root knot nematode *N. aberrans* has been found to be associated with numerous crops and native plants in temperate and sub-tropical regions of North and South America. Its presence in other parts of the world is not reported (EPPO, 2014).

Symptoms

Patches of poor growth are a common feature of affected crops. Above-ground symptoms include stunting, chlorotic leaves with rolled margins and wilting. Root galls are produced by the false root knot nematodes. Normally, the infected plants have few or no small feeder roots. Galls caused by *N. aberrans* can be mistaken for those caused by *Meloidogyne* species, but they differ in usually being more rounded and forming a rosary bead-like fashion (Fig. 7.8) and, hence, the



Fig. 78. Small, round, bead-like galls of *Nacobbus aberrans* on potato, *Solanum tuberosum*. (Photograph courtesy of J. Bridge, UK.)

common name of rosary-bead nematode or 'Rosario' is given to *N. aberrans*. Potato root galls generally contain only one female. Although it does not cause easily recognizable symptoms on potato tubers, the tubers have spongy tissue in the lenticels. *N. aberrans* usually penetrates the tubers to a depth of 1–2 mm below the skin (Manzanilla-López *et al.*, 2002).

Biology

N. aberrans second-stage juveniles emerge from the egg and invade small feeder roots. After several moults, they leave the root system as pre-adults (Manzanilla-López *et al.*, 2002). Under certain conditions, they remain in the root system in a quiescent stage for some time. Once the pre-adults become active, they invade the root system and produce small necrotic lesions prior to gall formation. The production of necrotic lesions by juvenile invasion is not as frequent as those caused by pre-adults. A portion of those that leave the root system become males. After the establishment of pre-adult females and gall formation, the nematodes develop to maturity.

Females often retain a portion of their eggs in their bodies in addition to depositing them in a gelatinous matrix outside the root. Pre-adults and juveniles also attack tubers, penetrating approximately 1–2 mm below the skin surface. There is no tuber galling or deformation associated with nematode infection. Depending on the host and temperature, generation time is usually between 25 and 30 days (Mai *et al.*, 1981). Under field conditions, there are three or four peaks of motile stages (Manzanilla-López *et al.*, 1998). False root knot nematodes are capable of withstanding low temperatures of up to -15°C . They can also survive in desiccated soil (Jatala and Kaltenbach, 1979).

Hosts

N. aberrans has a wide host range that includes more than 80 crop and weed species. Not all populations of *N. aberrans* are able to infect potato (Anthoine and Mugniéry, 2006; Del Carmen Tordable *et al.*, 2010; Lax *et al.*, 2011). *N. aberrans* can be separated into bean, potato and sugarbeet groups, and the existence of races has been suggested, with the populations of each group having distinct host preferences. At present, no system for race classification is in place (Lax *et al.*, 2011).

Survival and dissemination

Planting infested tubers, as well as the movement of infested soil that adheres to potatoes and farm implements are the major pathways of spread. Passive transport of the nematode with plants used as planting material, such as seed potatoes, probably contributes to spread (Jatala and de Scurrah, 1975). In Bolivia and Argentina, the nematode was the most frequently detected nematode in tubers, and more importantly, most seed tubers tested were infested by *N. aberrans* (Rojas *et al.*, 1998; Lax *et al.*, 2008).

Environmental factors

False root knot nematodes have a temperature range of $10\text{--}25^{\circ}\text{C}$, with temperature requirements differing among different populations of *N. aberrans* (Anthoine and Mugniéry, 2006). In the Andes, *N. aberrans* is associated with potatoes at temperatures of $15\text{--}18^{\circ}\text{C}$ (Mai *et al.*, 1981).

Periods of soil cooling and desiccation aid in the revival of nematode activity during spring, causing subsequent root infection (Jatala and Kaltenbach, 1979). *N. aberrans* is well adapted to survive extended periods of dry and cold periods and this, added to its ability to colonize many weeds, makes it one of the most difficult nematodes to manage.

Disease complexes

N. aberrans is often associated with species of *Meloidogyne* and *Globodera*, as well as other pathogens of potato such as *Synchytrium endobioticum* and *Spongospora subterranea* (Mai *et al.*, 1981). Simultaneous occurrence of these pests may cause disease complexes and higher levels of damage to potato.

Economic importance

N. aberrans plays an important role in reducing the yield of potatoes (Mai *et al.*, 1981; Ramos *et al.*, 1998; Manzanilla-López *et al.*, 2002; Lax *et al.*, 2011). It is considered to be the most important constraint to potato production in southern Peru and Bolivia (Mai *et al.*, 1981). In North America, it is reported in the USA and Mexico. In the USA, it parasitizes sugarbeet, other field vegetable and weed hosts, but not potato (Inserra, 1983). In Mexico, it causes economic loss in tomato, bean and chilli peppers.

Management measures

The use of clean planting material is the best way to prevent the spread of this nematode. Because of its extensive host range, control by crop rotation is difficult, although members of Gramineae and most of the Leguminosae are non-hosts (Mai *et al.*, 1981). Franco and Main (2006) found resistance in wild potato species against *N. aberrans*, which might be useful for breeding resistance. A range of crops and cultivars have been tested as potential trap crops for *N. aberrans*. Some oat cultivars and quinoa allowed invasion but no reproduction, and are therefore considered suitable as trap crop plants (Main *et al.*, 1999).

The fungus *P. chlamydosporia* is able to exert some level of control against the false root knot nematode (Pérez-Rodríguez *et al.*, 2007). Standard applications of commercial nematicide

formulations do not reduce populations of the nematode (Manzanilla-López *et al.*, 2002).

Diagnosis

Sampling and extraction of *N. aberrans* from soils and roots are similar to those described for root knot nematodes. The diagnosis of symptoms on roots can be problematic and often are mistaken for those caused by species of *Meloidogyne*. However, *N. aberrans* galls are characteristically formed on the lateral part of the roots and the galls often occur in a bead-like fashion (see Fig. 7.8).

According to Montalvo *et al.* (1992), one simple method to detect *N. aberrans* in fields is a bioassay consisting of growing a potato plant in moist soil maintained in a closed transparent container (e.g. plastic bag) kept at 25°C in darkness to allow the development of galls on the roots. Aktins *et al.* (2005) developed a molecular test for the detection of *N. aberrans*. As for all nematode detection methods, the problem of getting representative samples is a limiting factor in detecting low population densities that can already be damaging.

Ditylenchus

Potato tuber rot nematode *Ditylenchus destructor* and potato stem nematode *Ditylenchus dipsaci* have been reported from temperate climates, particularly eastern and western Europe. Both species also occur in North America and certain parts of South America (Mai *et al.*, 1981). Although the pest may be present, lack of reports on damage of potato caused by *Ditylenchus* spp. in the tropics (Njuguna and Bridge, 1998) supports the minor relevance of this pest outside the temperate zone.

Symptoms of damage

D. destructor mainly damages tubers, with the earliest below-ground symptoms being small, white, chalky or light-coloured spots just below the surface of the tuber. The symptoms become evident in the advanced stages of development, when the tuber surface is marked by sunken, dark-coloured pits or skin cracks (Mwaura *et al.*, 2015a). As the affected areas coalesce, tissue darkens and is invaded by bacteria and fungi.

More tuber tissue becomes damaged as populations increase. The nematode continues to live and develop in harvested tubers (Winslow, 1978b; Mai *et al.*, 1981).

D. dipsaci is mainly a parasite of the stem of many crop plants, where it attacks leaves and petioles, causing shortened, thickened and malformed foliage. However, this nematode also injures potato tubers, producing conical pits (Fig. 7.9) often accompanied by skin splitting (Mai *et al.*, 1981; Mwaura *et al.*, 2015a).

Biology

The morphology and biology of *Ditylenchus* species on potatoes follow the general patterns described for this genus (Chapter 2, this volume).

D. destructor has a wide host range and can survive on weeds and a wide range of soil-inhabiting fungi (Winslow, 1978b; Jensen *et al.*, 1979). It can also survive on infected tubers left in the field. The nematode will survive in soils at temperatures as low as -28°C . However, major infestation will occur at $15\text{--}20^{\circ}\text{C}$ and a rather high relative humidity of 90–100%. High relative humidity is a very important factor in the establishment of the nematode. The nematode cannot survive under drought or low (below 40%) relative humidity (Winslow and Willis, 1972; Winslow, 1978b; Jensen *et al.*, 1979).

Spread

Dissemination occurs by the introduction of infected tubers and in soil adhering to seed pieces



Fig. 7.9. Tuber damage caused by *Ditylenchus dipsaci* on potato, *Solanum tuberosum*. (Photograph courtesy of P. Mwaura, JKI, Germany.)

(Mai *et al.*, 1981). Irrigation water and cultivation by infested farm tools and machinery are other pathways for spread.

Economic importance and control

High yield losses occur in the areas where climatic conditions favour establishment of the potato rot nematodes. The effect of nematodes will manifest itself at harvest or storage, when infected tubers rot. The use of healthy tubers is the most effective measure in controlling the nematodes. Resistance against tuber rot and stem nematodes has been identified (Mwaura *et al.*, 2015b). The rotation of potatoes with sugarbeet and other non-host crops can reduce nematode populations (Winslow, 1978b) but may be limited because of the wide host range. Various cultural control programmes have contributed successfully to the management of these nematodes. Seed certification will continue to play a major role in reducing the impact of these nematodes.

Pratylenchus

Root lesion nematodes, *Pratylenchus* spp., are known to damage potatoes in the temperate, tropical and subtropical regions. *Pratylenchus crenatus*, *Pratylenchus minyus*, *Pratylenchus thornei*, *Pratylenchus scribneri*, *Pratylenchus brachyurus*, *Pratylenchus andinus*, *Pratylenchus penetrans*, *Pratylenchus coffeae*, *Pratylenchus vulnus* and *Pratylenchus flakkensis* are the most important species associated with potatoes (Jensen *et al.*, 1979; Mai *et al.*, 1981). *P. penetrans* is one of the major *Pratylenchus* species on potato in the temperate zone (Bélair *et al.*, 2006), but has also been reported on potato in the Philippines (Pedroche *et al.*, 2013).

High populations of lesion nematodes cause areas of poor growth, where plants are less vigorous and often turn yellow and cease to grow. Damage is often caused by direct feeding, and usually only cortical tissues are affected. Large nematode populations cause extensive lesion formation and root cortex destruction (Mai *et al.*, 1981). Tubers are often attacked and small lesions are formed on the surface. Infected tubers are sources of nematode inoculum and aid in the survival of the nematodes.

Because of their extensive host range, including weeds (Bélair *et al.*, 2007), crop rotations are often not effective and should be developed with caution. These nematodes interact with a series of pathogenic organisms in the development of disease complexes (Jensen *et al.*, 1979; Mai *et al.*, 1981).

Other nematodes of potatoes

Although many other nematodes are reported to cause damage to potatoes, few are of global concern. Other nematodes of potatoes in the tropics and subtropics are *Thecavermiculatus andinus*, *Trichodorus* spp. and *Paratrichodorus* spp. *Scutellonema bradys*, the yam nematode, has been reported to be damaging on potato, which is also a host for this nematode (Coyne *et al.*, 2011). *T. andinus* is an important nematode of potatoes in some Andean regions of Peru (Jatala, 1989). However, the extent of distribution and economic damage of this nematode to potatoes is not well documented. *Trichodorus* and *Paratrichodorus* spp. are of importance because of their involvement in the dissemination of potato viruses (Jensen *et al.*, 1979). In addition to their role in the transmission of viruses, they can also cause severe damage to the root system, leading to stunting and early senescence of the potato plant (Jensen *et al.*, 1979).

Sweet Potato

Sweet potato belongs to the series *Batatas* (Austin, 1978). Cultivated sweet potato is hexaploid and is closely related to the diploid wild relative *Ipomoea trifida* (Roullier *et al.*, 2013). Taxonomically the *I. batatas* complex includes *Ipomoea trifida*, *Ipomoea littoralis* and *Ipomoea leucantha*. Several other diploid and tetraploid *Ipomoea* species are used for breeding research (Yen, 1982). The wild species *Ipomoea triloba*, *I. trifida* and *Ipomoea grandifolia* have been targeted in breeding programmes for resistance to biotic and abiotic factors and enhancement of nutrient content and yield (Khoury *et al.*, 2015). High genetic diversity in sweet potato cultivars from different regions has been revealed using morphological (Tairo *et al.*, 2008) and molecular markers (He *et al.*, 2006; Elameen *et al.*, 2008; Veasey *et al.*, 2008).

Sweet potato is a perennial herb with vine-like habits and can be propagated vegetatively by

stem cuttings. The storage roots become swollen as the plant matures. Although sweet potato can perform well with limited inputs, the application of poultry manure and NPK fertilizer in combination with conventional tillage increases tuber yield (Agbede, 2010). It can withstand periods of irregular drought and rainfall (Horton *et al.*, 1984). Sweet potato ranks fourth and sixth on the list of dry matter production per hectare and edible energy production per hectare per day, respectively. Orange-fleshed sweet potato is an important source of vitamin A and has been used in combating vitamin A deficiency (Islam *et al.*, 2016).

Nematodes of Sweet Potato

Although a large number of nematode species are associated with sweet potatoes, only a few are of economic concern. The most important nematode genera attacking sweet potatoes are species of *Meloidogyne*, *Pratylenchus* and *Ditylenchus*, as well as *R. reniformis*.

Meloidogyne

Root knot nematodes, *Meloidogyne* spp., are widely distributed in the tropics, subtropics and warmer temperate regions of the world. The species *M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla* and *M. enterolobii* have been associated with sweet potato (Iwahori *et al.*, 2000; Iwahori and Sano, 2003; Dongro *et al.*, 2006; Musarrat *et al.*, 2006; Gao *et al.*, 2014), with *M. incognita* being considered as the most important species.

The distribution of *M. hapla* is limited to the cooler, temperate growing regions. *M. enterolobii* has been reported to infect sweet potato in China (Gao *et al.*, 2014) and Kenya (Karuri *et al.*, 2017a,b). It reproduces in the cultivars Beauregard, Covington, Evangeline, Hernandez and LA 05-111 (Brito *et al.*, 2014). Sweet potato is a non-host to certain isolates of *M. javanica* (Sano and Iwahori, 2002).

Symptoms

Meloidogyne species attack both roots and storage roots, causing swellings or galls of different shapes, but they fail to induce the prominent

galls on sweet potato roots as they do on many other crops. Sweet potato cultivars respond differently to *M. incognita* populations differing in virulence. If the initial nematode population is high, they cause a pruning effect, which can be overcome by vigorous growth and excessive lateral root production (Jatala, 1989). They also cause root tip necrosis in hypersensitive and resistant plants, while causing a somewhat general root necrosis in roots of susceptible cultivars. Physiological stresses associated with nematode parasitism can induce longitudinal cracking (Fig. 7.10) during development and swelling of the storage roots (Clark and Moyer, 1988). This root cracking can allow the establishment of secondary organisms and subsequent rotting (Lawrence *et al.*, 1986). The tubers may also have galls on the surface, which gives them a warty appearance (Fig. 7.11).

Females can be observed in the tissue on sliced storage roots and are usually associated with brown, necrotic cells around them. Infected plants exhibit general symptoms of damage associated with poor root growth, such as yellowing,

stunting, reduced fresh shoot and root weight, and the tendency to wilt during the warmer periods of the day. Infected sweet potatoes have abnormal flowering, reduced shoot:tuber ratio and an increase or decrease in the number of days taken by the tuber to flower (Agu, 2004). Areas in the field where there are infected plants have a patchy appearance.

Biology

The life cycle of *M. incognita* and other root knot species on sweet potato follows the general pattern specific to this genus (see Chapter 2, this volume). Feeder and storage roots are attacked at the same rate. The depth of penetration is dependent on the time of penetration of storage roots. *M. incognita* can complete several generations during the growing season of the crop dependent on the prevailing temperature (Jatala and Russell, 1972). *Meloidogyne* species develop well in light, sandy loam soils, which are often used for the cultivation of sweet potato.

The population density of root knot nematodes decreases when there is excess moisture,



Fig. 7.10. *Meloidogyne*-induced longitudinal cracking on tubers of sweet potato, *Ipomoea batatas*. (Photograph courtesy of H. Karuri, University of Embu, Kenya.)



Fig. 7.11. Galls of root knot nematode, *Meloidogyne* spp., on tubers of sweet potato, *Ipomoea batatas*. (Photograph courtesy of H. Karuri, University of Embu, Kenya.)

and high levels of infection can occur even when there is limited availability of water (Clark and Moyer, 1988). The number of female egg masses that are produced is dependent on the sweet potato cultivar (Sano and Iwahori, 2001). In susceptible cultivars, giant cells that are formed during *M. incognita* infection block the xylem and interrupt the flow of materials along the vessels (Wanderley and Santos, 2004). *Meloidogyne* juveniles and/or eggs can survive in storage roots. The nematode can also survive on many alternative weed hosts.

Spread

Root knot nematodes can be disseminated with infected root or soil. Irrigation water and unclean farm tools and machinery can also contribute to the dissemination of the nematodes. The nematode is not present in stems; therefore, the upper part of the sweet potato vine can be used as nematode-free planting material.

Economic importance

Meloidogyne species reduce plant growth and tuber yield. In South Africa, over an 11% decrease in the marketable yield due to a reduction in the storage roots has been reported (Kistner *et al.*, 1993). In Nigeria, tuber yield reductions of up to 20% after infection with *M. incognita* have been observed (Okechalu and Wonang 2015). Damage in the form of deep cracks (Fig. 7.10) greatly reduces the marketable value of sweet potato tubers and is of importance in assessing economic losses (Johnson *et al.*, 1992; Sharma *et al.*, 1997). Contributing to the economic importance is the fact that *Meloidogyne* infestations often lead to the establishment of disease complexes. Cracks in infected stored tubers enable the penetration and establishment of many secondary and/or pathogenic organisms such as *R. solanacearum* and *Fusarium* species.

Management measures

Successive planting of sweet potato for two successive seasons increases nematode population density in the soil (Johnson *et al.*, 1996). Crop rotations as a management measure for reducing root knot populations are not always effective, because of the extensive host range of *Meloidogyne*

species. However, the rotation of sweet potato with groundnuts and maize reduces *Meloidogyne* inoculum in soil (Reddy, 2015). *Crotalaria juncea* and *Crotalaria spectabilis* have shown some effect against *M. incognita* (and *P. coffeae*) in sweet potato fields in Japan (Torigoe, 1996).

Control of root knot in sweet potato by crop rotation with resistant cultivars is recommended (Fukunaga and Iwahori, 2002). Resistant cultivars produce a compound that reduces the penetration rate of *M. incognita* juveniles and decreases nematode development when penetrated (Jatala and Russell, 1972). In *I. trifida*, resistance to *M. incognita* is exhibited by a hypersensitive reaction whereby necrotic cells develop close to the point of nematode penetration (Komiya *et al.*, 2006). The resistant sweet potato cultivar Shinjani expresses a unique protein on infection with *M. incognita* (Lee *et al.*, 2012). Root knot nematode resistance in sweet potato is controlled by multiple genes (Mcharo *et al.*, 2005). Cultivars that are resistant to *Meloidogyne* species can be identified using molecular markers, such as randomly amplified polymorphic DNA (RAPD) or amplified fragment length polymorphism (AFLP) markers (Ukoskit *et al.*, 1997; Cervantes-Flores *et al.*, 2008).

Improved sweet potato cultivars with resistance to local *Meloidogyne* spp. have been bred for the arid and saline soils of northern Chile (Gallo *et al.*, 2001). Chen (1993) mentions resistant cultivars for China. Resistance at different levels has been found in numerous sweet potato cultivars in Brazil, Nigeria, Kenya, South Africa, Japan, South America and the USA, although resistance can vary with different populations of the nematode (Mohandas and Palaniswami, 1990; Mohandas *et al.*, 1996; da Silveira *et al.*, 1997; Vilmala and Rajendran, 1998; Freitas *et al.*, 2001; Sano *et al.*, 2002; Katayama *et al.*, 2003; Tamiya *et al.*, 2003; Marchese *et al.*, 2010; Bassey *et al.*, 2012; Nwankwo *et al.*, 2012; Gomes *et al.*, 2015; Lima *et al.*, 2016; Pofu *et al.*, 2016; Karuri *et al.*, 2017a,b). Other cultivars carrying various degrees of resistance to *Meloidogyne* spp., particularly to *M. incognita*, are mentioned by Sasser and Kirby (1979).

Cultivars Hernandez, Excel and Jewel are resistant to North Carolina populations of *M. incognita* race 3 and to *M. javanica*. These three cultivars, plus two others, Beauregard and Porto Rico, are also resistant to *M. arenaria* race 2

(Cervantes-Flores *et al.*, 2002a). The cultivars Supresa, Arroba, Pira 1 and Coquino plus 21 clones have also shown degrees of resistance to *M. incognita* (races 1, 2 and 3) and *M. javanica* in Brazil (Peixoto *et al.*, 1998). The virulence of nematode populations of the same host race varied among and within sweet potato genotypes, although several clones showed resistance to all North Carolina *Meloidogyne* populations tested, suggesting that different genes could be involved in the resistance of sweet potato to root knot nematodes (Cervantes-Flores *et al.*, 2002b).

Sweet potato cultivars with resistance to *M. incognita* and other major root knot species can still be infected with the highly aggressive *M. enterolobii* (Castagnone-Sereno, 2012). The use of resistant cultivars should be applied in combination with other management practices to prevent the selection of virulent *Meloidogyne* populations (Buena *et al.*, 2011).

Since sweet potato cultivation is generally characterized by low-input systems, the application of chemical control measures is usually cost prohibitive. Nevertheless, many organophosphates and carbamates are effective in controlling *Meloidogyne* species (Clark *et al.*, 1980; Gapasin, 1981; Reddy, 2015). In the USA, pre-plant nematicide treatments of soil infested with *M. incognita* doubled the yield of marketable sweet potato tubers and reduced the percentage of cracked tubers by over 40% (Hall *et al.*, 1988). A list of currently marketed nematicides is presented in Chapter 23, this volume. Soil solarization using clear polyethylene mulch reduces *M. incognita* populations at 0–30 cm soil depth and can be combined with organic material to increase efficacy (see Chapter 23, this volume).

Pasteuria penetrans, an obligate bacterial parasite of nematodes, has been used in Japan to control *M. incognita* on sweet potato. Soils treated with *P. penetrans* have lower populations of the nematode, and marketable yield is significantly higher (Tateishi, 1998; Tateishi *et al.*, 2007). This effect can be observed over a long period of consecutive cropping due to increased *P. penetrans* spore build-up. Soils with *P. penetrans* had significantly fewer *M. incognita* juveniles present in the seventh and eighth cropping cycles and increased marketable yields of tubers (Tateishi and Sano, 2001).

Although stem cuttings provide, in general, clean propagative material, several treatments

have been proposed for planting materials. Hot water treatment (Burk and Tennyson, 1941) and hot air treatment (Martin, 1962; Reddy, 2015) have been reported to be effective in eliminating *Meloidogyne* from root propagative material.

Diagnosis

Damage to roots can be assessed by rating the number of galls on roots and taking into account the amount of root necrosis. The degree of storage root infection can be determined by slicing the tuber roots at 0.5 cm thickness and observing the tissue for the presence of females. Staining techniques will aid in the detection of females with egg masses (see Chapter 2, this volume).

Molecular diagnosis of root knot allows accurate identification to species level and is important in designing nematode management strategies. Kiewnick *et al.* (2013) developed a multiplex PCR assay for the identification of *M. incognita*, *M. javanica* and *M. arenaria*. A multiplex PCR technique for simultaneous identification of *M. javanica*, *M. incognita* and *M. enterolobii* has also been developed (Hu *et al.*, 2011). Other methods of identification include mitochondrial haplotype-based techniques (Pagan *et al.*, 2015), use of LAMP, a loop-mediated isothermal amplification assay (Niu *et al.*, 2012), and HRM, a high-resolution melting curve analysis (Holterman *et al.*, 2012).

Rotylenchulus

R. reniformis, the reniform nematode, has been reported in south-eastern USA, as well as tropical and subtropical areas of the world where sweet potatoes are grown (Martin, 1960; Birchfield and Martin, 1965; Fassuliotis and Rau, 1967; Brathwaite, 1972, 1977; Gapasin and Valdez, 1979). It is commonly found on sweet potato in Japan and has been isolated from more than 40% of sweet potato fields in the northern part of Kyushu, and is considered to be a damaging pest of the crop in the area (Iwahori and Sano, 2003). Infestations of fields by *R. reniformis* and *M. incognita* in Papua New Guinea are considered to be part of the reason for sweet potato yield decline (Hartemink *et al.*, 2000). *R. reniformis* is the predominant nematode on sweet potato in Kerala, India (Ramakrishnan

and Mohandas, 1996), and it commonly occurs in mixed populations with other species on sweet potato in Egypt (Kassab and Taha, 1990). *R. reniformis* and *Rotylenchulus parvus* are associated with sweet potato in Uganda (Coyne *et al.*, 2003); the latter is also observed in sweet potato fields in South Africa (Marais and Swart, 2007). The species *Rotylenchulus variabilis* is common in sweet potato roots in Kenya (Njuguna and Bridge, 1998).

Infestation by *R. reniformis* can cause cracking of storage roots (Fig. 7.12). The induced cracks are deep and the exposed surfaces are healed over by the formation of callus and periderm. No juveniles or adults are found within the cracked sweet potato tubers. The population level necessary for cracking is considered to be very low. *R. reniformis* populations in the USA restricted storage root growth of a susceptible cultivar but not shoot growth. Root necrosis occurs and becomes more pronounced as the numbers of the nematode increase (Walters and Barker, 1994). At the point of *R. reniformis* infection, there is enlargement of phloem cells and compression of cambium and xylem cells (Yik and Birchfield, 1982). The formation of syncytia in sweet potato stele tissues also occurs during infection with *R. reniformis* (Vovlas *et al.*, 1985). Pericycle cells of susceptible cultivars that are infected with *R. reniformis* develop a thick wall that contains granulated cytoplasm (Cruz, 1988).

R. reniformis interacts with other pathogenic organisms, such as *Fusarium* spp., in the development of disease complexes. Interactions between *R. reniformis* and *M. incognita* have been reported whereby the reniform nematode became the predominant species in a sweet potato field, whereas

in greenhouse studies *M. incognita* became predominant in the concomitant infection of sweet potato (Thomas and Clark, 1983a,b).

Data on the control of these nematodes on sweet potatoes are rather limited. Birchfield and Martin (1968) demonstrated that, under field conditions, reniform nematodes could be controlled by in-row treatment with some nematicides. Some nematicides in the organophosphate and carbamate group also showed good control of nematodes, resulting in improved quality and yields of sweet potatoes. The use of nematicides in sweet potato plots infested with *R. reniformis* resulted in a reduction in the number of nematodes and an increase in sweet potato yield (Abel *et al.*, 2007). Other methods of controlling *R. reniformis* include hot water treatment of tubers, planting antagonistic plants, application of *P. lilacinum* to soil and use of resistant cultivars (Reddy, 2015).

Pratylenchus

The root lesion nematodes, *Pratylenchus* spp., most commonly associated with sweet potatoes are *P. brachyurus* and *P. coffeae*, causing necrotic lesions on both feeder and storage roots. In the presence of *M. incognita*, *P. brachyurus* causes a reduction in leaf growth (Agu, 2004). *Pratylenchus zeae* (Marais and Swart, 2001), *Pratylenchus goodeyi* (Coyne *et al.*, 2003) and *P. penetrans* (Inserra *et al.*, 2007) have also been observed in association with sweet potato in South Africa, Uganda and the USA, respectively. In Japan, around Kyushu, *Pratylenchus* spp. were found in 12–22% of sweet potato fields, with *P. coffeae* being the most predominant species (Iwahori *et al.*, 2000, 2001).

P. coffeae is thought to cause serious losses of sweet potato in Japan, and there have been breeding programmes to identify a source of resistance to the nematode (Marumine and Sakamoto, 1979). Sweet potato populations of *P. coffeae* from different regions of Japan exhibited different reproduction rates and amount of root damage, some being very virulent. Using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique revealed a distinct polymorphism and suggested the presence of more than two species of *Pratylenchus* (Mizukubo and Sano, 1997).

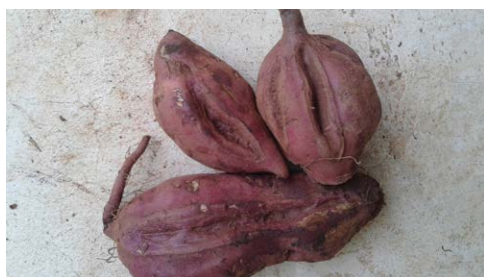


Fig. 7.12. Cracking caused by *Rotylenchulus reniformis* on tubers of sweet potato, *Ipomoea batatas*. (Photograph courtesy of H. Karuri, University of Embu, Kenya.)

Apparently, there is a certain degree of resistance to lesion nematodes in some of the existing sweet potato cultivars. Cultivars from the Cameroon highlands are poor hosts for *P. goodeyi* (Jacobsen *et al.*, 2009). Sweet potato cultivars from Vietnam (Nguyen *et al.*, 2015) and a cultivar from Japan (Uesugi *et al.*, 2008) have been reported as poor hosts for *P. coffeae*. Some local Peruvian cultivars with resistance to *M. incognita* are also known to exhibit resistance to another species, *P. flakkensis* (Anguiz and Canto, 1991).

Because of their relatively large host range, control measures against *Pratylenchus* spp., such as rotation, may not be very effective. The cultivation of palisade grass (*Brachiaria brizantha*) before planting sweet potato reduces the number of root lesions caused by *P. coffeae* (Uesugi *et al.*, 2015). The rotation of sweet potato with groundnut, soil fumigation and the addition of potassium-rich fertilizer, chicken manure and *P. lilacinum* in soil also reduce *Pratylenchus* spp. populations in soil (Reddy, 2015).

Ditylenchus

D. dipsaci, the stem nematode, and *D. destructor*, the potato rot nematode, are reported as serious pests of sweet potato in China (Jiang, 1990; Zhang, 1992; Wang and Zhao, 1994). They have also been reported in Uganda (Coyne *et al.*, 2003) and Kenya (Njuguna and Bridge, 1998).

Both nematode species cause a brown to black necrotic layer within the storage root, often leading to complete decay, especially following secondary invasion by pathogenic fungi such as *Fusarium oxysporum*, which has been observed in sweet potato infected with *D. destructor* (Zhang and Zhang, 2007). The presence of wounds in sweet potato increases the severity of infection by *D. destructor* (Xu *et al.*, 2015). Some

cultivars of sweet potato have been found to be resistant (Sun and Chen, 1994; Lin *et al.*, 1999; Yang *et al.*, 1999). In resistant cultivars, the xylem parenchyma cell walls are thicker and more lignified than in susceptible cultivars (Lin *et al.*, 1996).

Molecular markers linked to stem nematode resistance have been identified in sweet potato using RAPD (Zhou *et al.*, 2005) and AFLP markers (Qin *et al.*, 2009). Transgenic sweet potatoes with resistance to *D. destructor* have been developed by overexpression of the *IbMIPS1* gene (Zhao *et al.*, 2015), transformation of sweet potato using the *oryzacystatin-1* gene (Gao *et al.*, 2011) and expression of small interfering RNAs targeting the *unc-15* gene in the nematode (Fan *et al.*, 2015).

Control of *Ditylenchus* spp. is through the use of nematicides, soil fumigation and control of weeds, which act as hosts for the nematode (Reddy, 2015).

Other nematodes

Other nematodes of possible importance to sweet potato production when present in large populations are *Paratrichodorus* spp., *B. longicaudatus*, *R. similis*, *Helicotylenchus* spp. and *Scutellonema* spp. In pot experiments in India, *R. similis* caused 72–84% reduction in the weights of sweet potato roots at an initial density of 10,000 nematodes/plant; the economic threshold level is said to be 100 nematodes/plant (Koshy and Jasy, 1991).

The nematode genera *Criconemella*, *Hoplolaimus*, *Paratylenchus*, *Filenchus*, *Hemicriconemoides*, *Hemicycliophora*, *Trichodorus*, *Paratrichodorus*, *Rotylenchus*, *Scutellonema*, *Tylenchorhynchus*, *Tylenchus* and *Xiphinema* are also found in association with sweet potato (Coyne *et al.*, 2003; Marais and Swart, 2007; Karuri *et al.*, 2017a,b).

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8 Nematode Parasites of Tropical Root and Tuber Crops (Excluding Potatoes)*

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Root and tuber crops all produce starchy storage organs that are modified stems or roots, generally referred to as rhizomes, corms or tubers. They are the most important food commodities produced in many subtropical and tropical countries and are second only to cereals in the total world supply of carbohydrates. In addition to *Solanum* potato (*Solanum tuberosum*) and sweet potato (*Ipomoea batatas*) (see Chapter 7, this volume), the other most widely grown root and tuber crops are cassava (*Manihot esculenta*), yams (*Dioscorea* spp.), taro (*Colocasia esculenta*) and tannia (*Xanthosoma* spp.). A further 27 locally important root and tuber crops have been described (Kay, 1987).

The origin and history of root and tuber crops are well documented (Coursey and Haynes, 1970; Coursey and Booth, 1977; Leon, 1977). However, while the actual contribution of these crops to the world's food supply is substantial, they are generally poorly understood.

Cassava

M. esculenta is a perennial woody shrub of the Euphorbiaceae family. From its centre of origin in the Americas (Cock, 1984), cassava spread

first to Africa and then to Asia. In Africa, separate introductions were made into the west, first through the Congo Basin, and the east (Jones, 1959). Cassava cultivation is limited primarily to the tropics and subtropics, where it can be planted at any time of the year providing there is sufficient moisture for stem cuttings to take root. It has the ability to produce economic yields under relatively marginal soil and rainfall conditions, and has essentially been recognized as a small farm and subsistence crop, requiring minimal cash input for production. However, it is being cultivated on increasingly larger scales, both as a food crop, for fodder and also for industrial starch or ethanol (<http://www.cassavabiz.org/>; accessed 17 November 2017). Nigeria produces more cassava than any other country, while Thailand is the biggest exporter of the crop (FAOSTAT, 2014).

The enlarged storage roots have hydrocyanic glycosides in varying quantities, depending on age, cultivar and environmental conditions (Nartey, 1977). Cultivars are customarily designated as either a sweet or bitter type, which has purportedly been related to their cyanogenic glucoside content. However, analysis of various parts of the plants of bitter and sweet cultivars shows comparable levels (Nartey, 1977). Although it is grown principally for its swollen

*A revision of the chapter by J. Bridge, D.L. Coyne and C.K. Kwoseh in the second edition.

roots (storage roots), cassava leaves are also eaten, particularly in parts of Africa and especially in the countries of the Congo Basin.

Nematodes of Cassava

Cassava is regularly considered as being unaffected by nematodes, which is not the case and it can be heavily damaged. As with many tropical crops, a wide range of nematode species are recorded from cassava, which are presented in various reports, the most comprehensive of which include those by Hogger (1971), Caveness (1980), McSorley *et al.* (1983b), Ray *et al.* (1992), Coyne *et al.* (2003) and Rosa *et al.* (2014). Although the list of nematodes is extensive, the majority of the nematode species appear of limited importance, with little evidence of significant effect on the crop. The plant parasitic nematodes recorded most frequently with cassava are *Meloidogyne* spp., *Pratylenchus brachyurus*, *Rotylenchulus reniformis*, *Helicotylenchus erythrinae* and *Helicotylenchus dihystrera*. Of these, *Meloidogyne* spp. and *P. brachyurus* are most associated with damage to cassava. Most of these nematodes may interact with other pathogenic organisms in the development of disease complexes. Most data on nematodes of cassava are related to diagnostic and distribution studies, with some information from screening studies and a limited amount from pathogenicity work.

Meloidogyne species

Root knot nematodes are by far the nematodes most commonly associated with cassava. They have been reported on cassava across Africa, Asia, the Pacific and the Americas, including the USA. *Meloidogyne incognita* and *Meloidogyne javanica* are the most important, although *Meloidogyne arenaria* and *Meloidogyne hapla* are also reported (de Tanaka *et al.*, 1979; Coyne *et al.*, 2003) but do not appear to be of major concern. The aggressive species *Meloidogyne enterolobii* has also been identified on cassava in Brazil (Rosa *et al.*, 2014), raising the possibility that it also infects cassava elsewhere, while a range of other species have been tentatively identified infecting cassava (Coyne *et al.*, 2005a). In addition, numerous studies

make reference to *Meloidogyne* sp. because either identification to species has not been attempted or because the nematodes do not conform to the specifications of identified species. It is likely, therefore, that a greater range of species attack cassava. As with many crops, different *Meloidogyne* species can often occur in combination in the same location.

Symptoms of damage

The typical knotting of the feeder and fine filamentous roots is common and is the most obvious feature of *Meloidogyne* spp. infection (e.g. Bridge *et al.*, 1991). However, the naturally 'knobbly' and rough texture of the feeder roots can disguise nematode damage (Coyne, 1995) (Fig. 8.1). The long duration over which cassava can remain in the ground and the common 'piecemeal' method of harvesting also mean that nematode-affected root systems may decompose in the ground, so are not observed at harvest. This may contribute somewhat to the concept that nematodes are not an issue on cassava. Galling damage can vary considerably, but can be serious. Less common and rarely documented is damage to the storage roots themselves, such as the surface 'bubbling' observed in Uganda in farmers' fields and in Mozambique (Fig. 8.2) on some germplasm lines (~1%) in a breeder's selection trial (Coyne *et al.*, 2004). *M. incognita*, *M. javanica* and an unidentified *Meloidogyne* sp. were recovered from the cassava tissue. In some cases, the surface can be flaky in appearance, with high levels of necrosis apparent under the surface when thin sections are cut away (Fig. 8.3). Otherwise, *Meloidogyne* spp. associated with cassava concern solely the feeder (and fine) roots, with no evidence of damage to storage roots (Caveness, 1981; Coyne and Talwana, 2000; Makumbi-Kidza, 2001). Root knot nematodes do not appear to be directly related to rotting of storage tubers, but their presence is related to higher levels of tuber rot by fungal and bacterial pathogens (Akinlesi, 2014). Above-ground symptoms of *Meloidogyne* spp. damage are not normally obvious. Under light infection, increased aerial growth has been recorded (Caveness, 1982) and plant height observed to be unaffected following inoculation with *M. incognita* (Makumbi-Kidza *et al.*, 2000). Stem height and weight reduction (Gapasin, 1980,



Fig. 8.1. Galling of individual cassava roots infected with *Meloidogyne incognita*. (Photograph courtesy of D. Coyne.)

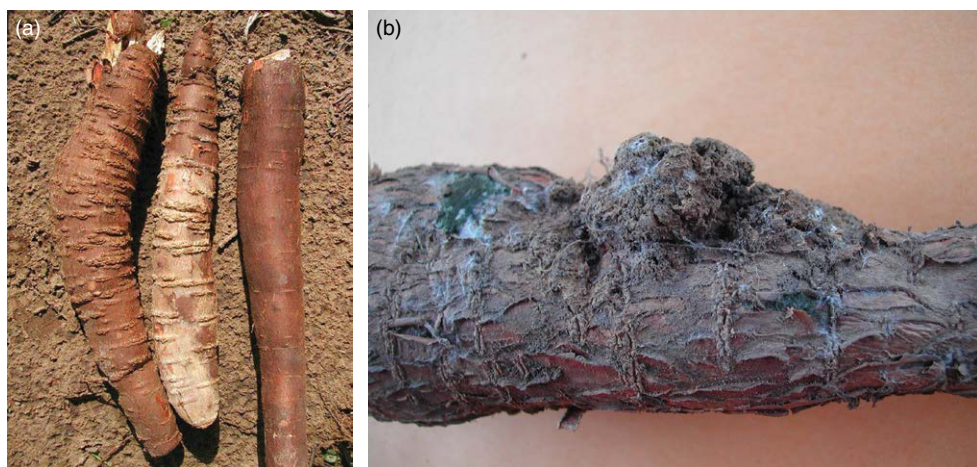


Fig. 8.2. Deformed and knobby cassava storage root in Mozambique (a) and Uganda (b) due to feeding of *Meloidogyne incognita*. (Photograph courtesy of D. Coyne.)



Fig. 8.3. Subcortex necrosis of cassava storage root due to feeding of *Meloidogyne incognita* in Mozambique. (Photograph courtesy of D. Coyne.)

1981; Caveness, 1981, 1982; Talwana *et al.*, 1997a) and reduced sprouting and establishment of cuttings, however, are associated with high *Meloidogyne* population densities (Talwana *et al.*, 1997a; Makumbi-Kidza *et al.*, 2000). In Thailand, early wilting of cassava leaves was associated with *Meloidogyne* infection (Chinasri *et al.*, 2014). In pots, inoculation with *M. incognita* reduced plant height by 35%, shoot weight by 30% and tuber weight by 54% (Akinlesi, 2014). In areas of Uganda, dead and dying cassava plants were associated with severe levels of *M. incognita* infection (Bridge *et al.*, 1991).

Although cassava contains cyanogenic glucosides, which probably form an element of the overall plant defence mechanism, there is little

evidence to suggest that they are related to nematode damage or defence. In one study, root knot infection on 11 cassava cultivars was unrelated to cyanide content (de Freitas and de Moura, 1986). However, Makumbi-Kidza (2001) found that two of ten clones assessed had higher cyanogenic potential in storage roots in *M. incognita*-inoculated soil compared with non-inoculated soil. The remaining eight clones were unaffected. *M. incognita* egg mass formation on the feeder roots of selected clones was, however, correlated negatively to the cyanogenic potential of the mother clone storage roots.

Disease complexes

There is relatively little documented evidence that root knot nematodes form associations with other pests or pathogens on cassava. Galling and mechanical damage of roots by nematodes will undoubtedly facilitate the entry and development of secondary pathogens, leading to increased levels of root necrosis and reduced root weights, compared with uninfected plants, as observed in some studies (Gapasin, 1980; Crozzoli and Hidalgo, 1992; Talwana *et al.*, 1997a; Coyne and Talwana, 2000; Akinlesi, 2014). In Uganda, Bridge *et al.* (1991) associated a possible secondary fungal root rot with severe nematode infestation in farmers' fields. The extent to which disease complexes occur, however, has been little investigated, and information is generally scarce, although nematode-infected roots are reportedly more susceptible to rot organisms (Théberge, 1985). The interaction between *M. incognita* and *Botryodiplodia theobromae*, one of the main causal agents of root rot in Nigeria (Dixon *et al.*, 2003), showed that their combined effects increased tuber rot by 48.1% across cultivars in Nigeria, most notably when nematodes were inoculated prior to the fungus (Akinlesi, 2014).

Economic importance

Under certain circumstances, root knot nematodes can be serious pests of cassava. However, while numerous pot and microplot studies have clearly demonstrated their highly pathogenic nature on cassava (e.g. Caveness, 1981; Crozzoli and Parra, 1999; Coyne and Talwana, 2000; Makumbi-Kidza *et al.*, 2000), data on their

economic impact are scarce and contradictory. Caveness (1982) showed that *Meloidogyne* spp. could cause 87% yield loss under heavy attack, with losses as high as 98% recorded in experimental plots (Théberge, 1985). However, evidence supporting similar or consistent levels of damage under farm conditions is limited. The combined analysis of data from four trials in Kenya and Uganda showed that galling intensity, *Pratylenchus* spp. and *Meloidogyne* spp. density were generally correlated negatively with both total and marketable yield (Coyne *et al.*, 2006). In farmers' fields, severe levels of galling damage have been observed in Uganda (Bridge *et al.*, 1991; Coyne and Namaganda, 1994). Coyne and Talwana (2000) later correlated root galling damage (cv. Ebwataneraka) in farmers' fields with reduced yield; by extrapolating data (albeit crudely), Coyne (2004) estimated that 17% of Uganda cassava producers were being subjected to 66% yield losses due to root knot nematodes. In field trials, solarization to reduce root knot nematodes in Nigeria resulted in 25 to >200% (depending on the cultivar and trial) increase in cassava yields, with nematode populations reduced by up to 73% and galling index by 70% (Abidemi, 2014). In pot studies, Crozzoli and Parra (1999) established that the tolerance limit for aerial dry weight and root fresh weight on cv. Tempranita slightly affected by *M. incognita* race 2 was 1.0 juvenile/ml of soil. *Meloidogyne* spp. damage to cassava appears to be most important, in terms of crop yield response, at or before tuber initiation (Makumbi-Kidza *et al.*, 2000), a period when the crop is also most vulnerable to water stress (Ekanayake *et al.*, 1998). Therefore, it is possible that *Meloidogyne* spp. infection occurring after tuber initiation may lead to visually detectable galling damage, but not to yield reduction, and may explain the difficulty in relating galling damage to yield from the field. Makumbi-Kidza *et al.* (2000) also indicated that production loss by *M. incognita* was through a reduction in storage root number as opposed to a reduced weight of individual storage root.

In addition to the direct losses of both quantity and quality of the cassava crop, there is the added effect of reduced stem height and weight associated with high *Meloidogyne* densities (Gapasin, 1980, 1981; Caveness, 1981, 1982; Chinasri *et al.*, 2014). This decreases available

leaf production, as well as the quality of the planting material available for the following season. Furthermore, the presence of *Meloidogyne* spp. at planting can suppress the sprouting of cuttings (Talwana *et al.*, 1997a; Makumbi-Kidza *et al.*, 1999), indicating that yields can be severely reduced through the prevention of emergence.

Management measures

Considering the limited understanding and awareness of the damage that *Meloidogyne* spp. can cause to cassava in the field, there has been little focus on their management, with the exception of varietal screening studies. The utilization of resistant cultivars on an international and national basis appears to be the most realistic and economical means of nematode management. However, in localized situations, particular management practices such as rotation systems, intercropping, fallowing, mulching and the use of nematicidal or antagonistic cover crops and by-products may be appropriate. Cassava cultivars differ considerably in their response to root knot nematodes (e.g. Da Ponte *et al.*, 1980; Caveness, 1981, 1982; Saka, 1982; Nwauzor and Nwankwo, 1989; Crozzoli and Hidalgo, 1992; Coyne and Talwana, 2000; Makumbi-Kidza, 2001; Coyne *et al.*, 2004; Udo *et al.*, 2008; Abidemi, 2014). Some cultivars have been recorded as immune, while others are highly susceptible. The *Meloidogyne* species screened against, however, has not always been identified, or has involved concomitant species. Differences in the reaction of cultivars is no doubt due not only to the different species, races or pathotypes of *Meloidogyne*, including combinations of species/pathotypes in the same location, but also to different population densities (McSorley *et al.*, 1983b). Caveness (1980), when screening cassava lines in Nigeria, found *M. incognita* more aggressive than *M. javanica*. However, cassava cultivars had variable and differential response to the two nematode species in Uganda, while a combined inoculation increased damage (D.L. Coyne, unpublished data), indicating that individual and combined resistance screening is recommended. Several studies have also focused on improving the efficiency and ease of cassava screening procedures (Talwana *et al.*, 1997b; Vuuren and Woodward, 2001; Kagoda *et al.*, 2004; Coyne *et al.*, 2012).

Although yield increases have been obtained in Latin America with nematode control following soil fumigation (Da Ponte and Franco, 1981), the economic value of this is questionable (Hillocks and Wydra, 2002). Gapasin (1981) reported yield increases following the pre-plant application of nematicides, while Diomandé (1982) obtained no yield improvement following fumigation with dibromochloropropane (DBCP) to control *M. javanica* in Côte d'Ivoire. Cassareep, a by-product of the cassava industry, was apparently effective in controlling *M. incognita* and *M. javanica* on cassava (Da Ponte and Franco, 1981). In field trials, solarization reduced root knot densities significantly, resulting in increased yields, which were improved greatly by mulching with *Tithonia diversifolia* (Abidemi, 2014). The application of microbial beneficials, such as the arbuscular mycorrhizal fungi *Glomus mosseae* or the nematophagous fungi *Purpureocillium lilacinus* (syn. *Paecilomyces lilacinus*) to root knot-infected cassava in pots proved very effective in reducing nematode densities and galling damage and increasing yields, indicating their potential for protecting and improving cassava production (Akinlesi, 2014). It is important to note that with more intensified and monoculture cassava production, the importance of nematodes as production constraints is likely to become more prominent.

Pratylenchus species

P. brachyurus is probably the second most important nematode parasite of cassava after root knot. It occurs on the crop around the world (McSorley *et al.*, 1983b). Other lesion nematodes have been associated with cassava, but not nearly to the extent of *P. brachyurus*. *Pratylenchus pseudopratensis* is found in Nigeria (IITA, 1978), *Pratylenchus zae* in the Philippines (Timm, 1965) and Brazil (Rosa *et al.*, 2014) and *Pratylenchus coffeae* in Java (de Fluiter and Mulholland, 1941, in McSorley *et al.*, 1983b) and Nigeria (D.L. Coyne, unpublished). Cassava is an excellent host for the nematode and *P. brachyurus* is the most common nematode occurring on cassava in Togo and Amazonia, Brazil (De Guiran, 1965; Rosa *et al.*, 2014). In Togo, the nematode was attributed to a gradual yield decline over several years, where population densities up to

400/g of root were observed. In a field experiment at the same location, soil fumigation with DBCP improved yield by 8.5% for aerial growth and 7.9% for storage roots. In a greenhouse experiment in Brazil, an eightfold population increase in *P. brachyurus* density was observed after 3 months on cv. IAC-105.66 (Charchar and Huang, 1981). Zem (1979), however, reported that *P. brachyurus* caused no obvious damage to the crop in Brazil. Considerable variability in the reaction of cassava cultivars to *P. brachyurus* (Luc, 1971; Corbett, 1976) indicates that the management of *P. brachyurus* may be possible through the use of tolerant or resistant cultivars. De Guiran (1965) described 42 cultivars as highly susceptible and resistant based on *P. brachyurus* development after 3 months. Rosa *et al.* (2014) found two cultivars, Caipora and Colonial, resistant to *P. brachyurus*, immune to *P. zeae*, but susceptible to *M. incognita* race 3.

Other nematodes of cassava

Despite the frequent occurrence of many other nematode species on cassava, there is little evidence of economic damage being caused by them. Some commonly occurring nematodes, such as *Aphelenchoides* spp. and *Aphelenchus avenae*, are viewed primarily as fungivorous, and their presence in high densities around plant roots related more to fungal contamination of the roots, as observed by Bridge *et al.* (1991), or as part of the decomposition process. *R. reniformis*, although regularly associated with cassava, was found to decline under cassava (McSorley *et al.*, 1983b). *Scutellonema bradys*, a key pest of yam, is regularly recovered from cassava in West and Central Africa (e.g. Luc and de Guiran, 1960; Caveness, 1967b; Addoh, 1971; D.L. Coyne unpublished), but with little indication of damage. In pot assessments in Nigeria, however, inoculated cassava had reduced root weights, which became necrotic compared with the controls (Fig. 8.4). It is possible that such damage is being overlooked in the field, as *S. bradys* is obviously able to infect and damage cassava. Cassava is described as an excellent host for *Scutellonema clathricaudatum*, along with *Helicotylenchus microcephalus* (Caveness, 1967a). In Uganda, cassava was host to at least six species of *Scutellonema* (*Scutellonema brachyurus*,



Fig. 8.4. Cassava roots infected with *Scutellonema bradys* (left) compared with healthy roots from a pot inoculation study (right). (Photograph courtesy of D. Coyne.)

S. clathricaudatum, *Scutellonema magniphasma*, *Scutellonema paralabiatum*, *Scutellonema unum* and *Scutellonema* sp.) (Coyne *et al.*, 2003), and to four in Nigeria (*Scutellonema aberrans*, *S. bradys*, *Scutellonema cavenessi* and *S. clathricaudatum*) (F.E. Caveness, unpublished), but without any obvious damage recorded. In exploratory work in Cameroon, over 10% of fields surveyed contained *Heterodera* spp. juveniles from soil around cassava roots (Tambe, 1999), while a small number of cassava fields also contained *Heterodera* spp. juveniles in a survey in the Democratic Republic of Congo (D.L. Coyne, unpublished).

Yams

Yams, *Dioscorea* spp., are among the oldest food crops known to humans (Alexander and Coursey, 1969), and globally the fourth most important root and tuber crop. Their large-scale cultivation as a food crop is concentrated mostly in three main areas of the world: West Africa, the Pacific area (including Japan) and the Caribbean, but is also locally important across Africa and tropical America. An estimated 91% of world production (~68 Mt) is produced in West Africa (FAOSTAT, 2014), where they represent key sociocultural symbols, steeped in traditional rituals and religions.

The genus *Dioscorea* consists of over 600 species, of which 13 are important food crops: *Dioscorea rotundata*, *Dioscorea cayenensis*,

Dioscorea dumetorum, *Dioscorea hispida*, *Dioscorea alata*, *Dioscorea esculenta*, *Dioscorea bulbifera*, *Dioscorea opposita*, *Dioscorea japonica*, *Dioscorea trifida*, *Dioscorea nummularia*, *Dioscorea transversa* and *Dioscorea pentaphylla* (Malaurie *et al.*, 1998). A number of *Dioscorea* species are also grown commercially as a source of diosgenin, cortisone and steroidal hormones, used in the manufacture of oral contraceptives, sex hormones and cortisone, and others for use in desserts, ice cream and other processed foods (Lebot, 2009).

Some yams produce single, large tubers, while others produce many small tubers. Yams can also form bulbils in the leaf axils, as in *D. bulbifera* and some cultivars of *D. rotundata* and *D. alata*. Most yams have good storage qualities and can survive for periods of 3–4 months or longer. Therefore, they are relied upon for local food security and income generation. Yams are normally propagated vegetatively from whole, small tubers (seed tubers/seed yams), portions of tubers (setts) or bulbils. The small seed tubers can be formed by cutting and removing the main tuber during the growing season. They can also be produced by the use of ‘mini-setts’ or ‘microsetts’ cut from tubers, vine pieces or *in vitro* tissue culture plantlets (Jova *et al.*, 2012; Dibi *et al.*, 2016). Yams are often monocropped, but can be intercropped. In rotation systems, yam is traditionally planted first following forest clearance or long-term fallows (Carsky *et al.*, 1999).

Nematodes of Yams

Many different nematode species have been found to be associated with yams. The nematodes of particular importance are endoparasites of roots and tubers. Those known to cause serious damage by mainly reducing tuber yield and quality are *S. bradys*, *P. coffeae*, *Pratylenchus sudanensis* and *Meloidogyne* spp.

Scutellonema bradys

The yam nematode *S. bradys* is the cause of a decay of yam tubers known as ‘dry rot disease’. It is found in many yam-growing areas of the world, having been reported from West Africa

(Benin, Burkina Faso, Côte d'Ivoire, the Gambia, Ghana, Guinea, Mali, Nigeria, Senegal and Togo), East and Central Africa (Cameroon, Kenya, Sudan and Tanzania), Central America and the Caribbean (Barbados, Costa Rica, Cuba, Dominica, Dominican Republic, Guadeloupe, Guatemala, Haiti, Jamaica, Martinique, Puerto Rico and Trinidad and Tobago), South America (Brazil and Venezuela) and Asia (India and Pakistan) (Bridge *et al.*, 2005; CABI datasheet, 2016).

Symptoms of damage

Dry rot associated with *S. bradys* occurs in the outer 1–2 cm of tubers. In the initial stages, cream or light yellow lesions occur below the outer skin of the tuber (Fig. 8.5). There are no external symptoms at this stage. As the disease progresses, it spreads into the tuber, normally to a maximum depth of 2 cm, but sometimes deeper. In these later stages, infected tissues become yellow or brown and then turn dark brown to black (Fig. 8.6). External cracks can appear in the skin of the tubers (Fig. 8.7) and parts can flake off, exposing patches of dark brown, dry rot tissues. Symptoms of dry rot are typically associated with nematode damage and are correlated strongly with *S. bradys* densities in the tuber (Kwoseh, 2000), but occurrence of cracks in the absence of *S. bradys* has been reported (Baimey *et al.*, 2009; Coyne *et al.*, 2012) and therefore may be an unreliable indicator for the presence of or damage by *S. bradys*. The most severe symptoms of dry rot are seen in mature tubers, especially



Fig. 8.5. Lesions caused by *Scutellonema bradys* in the outer subsurface part of the yam (*Dioscorea rotundata*) tuber. (Photograph courtesy of D. Coyne.)



Fig. 8.6. Dry rot disease caused by *Scutellonema bradys* in the outer part of the yam (*Dioscorea rotundata*) tuber. (Photograph courtesy of D. Coyne.)



Fig. 8.7. External cracks on a yam (*Dioscorea rotundata*) tuber (left) caused by *Scutellonema bradys* compared to an uninfected tuber. (Photograph courtesy of D. Coyne.)

during storage, when it is often associated with the general decay of tubers. Dry rot, however, can also develop to quite an advanced stage without being visually obvious, causing deterioration of the tissue underneath an intact periderm of an apparent healthy tuber. Only once the surface is removed with a knife or thumbnail is the underlying damage revealed.

No foliar symptoms have been observed on yams growing in soil infested with *S. bradys*.

Biology and life cycle

S. bradys is a migratory endoparasite present in yam soils, roots and tubers. It is a vermiform nematode when mature, measuring about 1 mm in length, and has a well-developed stout stylet

for puncturing cells. All active stages are infective. It invades young, developing tubers through the tissues of the tuber growing point, alongside emerging roots and shoots, through roots and also through cracks or damaged areas in the tuber skin (Bridge, 1972).

Nematodes feed intracellularly in tuber tissues, rupturing cell walls and causing the formation of cavities (Goodey, 1935; Bridge, 1973; Adesiyan *et al.*, 1975a). They are confined mainly to the subdermal, peridermal and underlying parenchymatous tissues in the outer 1–2 cm of tubers. *S. bradys* continues to feed and reproduce in yams stored after harvesting. Population densities can increase substantially over a 5- to 6-month storage period. In tubers with partial dry rot, more nematodes are found in the oldest apical portions, adjacent to the stems (Adesiyan, 1977). In Martinique, the highest multiplication rates occurred within the tuber after harvest and coinciding with the initiation of tuber dormancy (Cadet and Quénéhervé, 1994). Nematode densities were found to decrease after 5 months of storage though, likely due to deterioration of the tubers (Baimey *et al.*, 2009).

S. bradys is morphologically similar to *S. cavenessi* and *S. clathrcaudatum*, which may all be synonymous with each other (Baujard and Martiny, 1995). Molecular assessment of *S. bradys* from Nigeria and Costa Rica has shown substantial polymorphic variation between different populations and between individuals within a population but which is not necessarily indicative of pathogenic variability (Coyne *et al.*, 2012; Humphreys-Periera *et al.*, 2014). In Benin, molecular assessment of *S. bradys* populations, based on ITS1 rDNA and COI mtDNA, revealed no genetic separation between populations, and no clear subgroups (Etchiha Afoha *et al.*, 2016).

Survival and dissemination

No true survival stage is known with *S. bradys*, but populations are maintained in the absence of yams, probably on other host plants. Sizeable densities of the nematode can be found in soil at the beginning of the yam growing season (Obigbesan and Adesiyan, 1981; Adesiyan and Badra, 1982; Opperman *et al.*, 2016).

Yam planting material provides the principal means of *S. bradys* dissemination. Tubers retained from the harvest for the next planting

season with comparatively low nematode densities do not produce external symptoms (Bridge, 1973), creating the continued risk of dissemination and perpetuating the disease cycle.

Environmental factors affecting parasitism

Nematodes in stored tubers are affected by storage conditions. Populations of *S. bradys* increase at twice the rate in tubers stored at 22–32°C and relative humidity of 40–85% compared with those in tubers stored at 16–18°C and relative humidity of 80–85% (Adesiyani, 1977).

Other hosts

Commonly grown food yams are all hosts of *S. bradys* and susceptible to dry rot disease (see Bridge *et al.*, 2005). Wild *Dioscorea* spp. in Nigeria and Cameroon support *S. bradys* that cause dry rot in tubers (Bridge, 1982; Bridge *et al.*, 1995). Also, the wild yam *Dioscorea praehensilis*, from the Republic of Guinea is reported to be susceptible to *S. bradys* (Kwoseh, 2000). There are many other crop and weed hosts of *S. bradys* (Adesiyani, 1976; Coyne *et al.*, 2011; Claudius-Cole and Fawole, 2016), but most plants are relatively poor hosts in comparison with yams. Sesame and cowpea support high root densities, and melon can increase soil densities, but the cultivar is important. *S. bradys* also occurs on other root and tuber crops, such as cassava (Coyne *et al.*, 2006), *Xanthosoma* sp., taro and sweet potato (*I. batatas*), which did not appear to be particularly good hosts (Kermarrec *et al.*, 1988). However, differential host assessments in Nigeria found potato (*S. tuberosum*) to be a good host (Coyne *et al.*, 2011), as well as cassava and sweet potato (D.L. Coyne, unpublished data). *Crotalaria ochroleuca*, *Crotalaria juncea* and *Lablab purpureus* were as similarly supportive of *S. bradys* infection as cowpea (Claudius-Cole and Fawole, 2016).

Disease complexes

Dry rot disease can be caused by *S. bradys* in the absence of other organisms (Bridge, 1973; Adesiyani *et al.*, 1975a), although it has been suggested that the disease is caused by a bacterium, *Corynebacterium* sp., in association with

S. bradys, which acts as a wounding agent (Ekundayo and Naqvi, 1972). The more extensive internal decay of tubers known as 'wet rot', 'soft rot' or 'watery rot' is associated with fungal and bacterial pathogens (Adeniji, 1970; Ogundana *et al.*, 1970; Ekundayo and Naqvi, 1972). This general decay of tubers, which is a serious problem in stored yams, is amplified when tubers are wounded or damaged (Adeniji, 1970; Ogundana *et al.*, 1970). The damage caused by nematodes can predispose tubers to invasion by decay organisms, resulting in complete rotting of the tubers (Goodey, 1935). The principal fungi causing internal tuber decay are *B. theobromae* and *Fusarium* sp., although other fungi and the bacterium *Erwinia* sp. are frequently isolated from decaying tissues (Coursey, 1967; Adeniji, 1970; Ogundana *et al.*, 1970; Ekundayo and Naqvi, 1972; Moura *et al.*, 1976; Demeaux *et al.*, 1982; Ogaraku and Usman, 2008). Nematodes and fungi are found together in the transitional stage between dry rot and wet rot, but nematodes do not occur in the 'late wet rot' stage deep in the tubers (Adesiyani *et al.*, 1975a).

In West Africa, root knot galling and dry rot symptoms were both observed on the same yam tubers in fields, markets and farmers' stores (Affokpon *et al.*, 2015). In the West Indies, *S. bradys* infrequently occurs together in the same tubers with *P. coffeae*; infection by one species only is more usual, however. The establishment of one species in tuber tissues apparently prevents concomitant infection by the other species (Castagnone-Sereno and Kermarrec, 1988). When both species are present, *P. coffeae* dominates over *S. bradys* (Acosta and Ayala, 1976a). Consequently, *S. bradys* is viewed as less of a yam problem in Caribbean islands such as Martinique and Guadeloupe, where it is displaced by *P. coffeae* (Bridge *et al.*, 2005).

Economic importance

The primary importance of *S. bradys* is in the direct damage it causes to tubers. Weight differences between healthy and diseased tubers harvested from the field have been estimated to be 20–30% in Côte d'Ivoire (Smit, 1967, in Bridge, 1982), 0–29% in Nigeria (Wood *et al.*, 1980) and 0–52% in Benin (Baimey *et al.*, 2009). Treatment of seed setts naturally infected with *S. bradys* led to a doubling of tuber yields in

Nigeria, which were also healthier and stored better (Kenyon *et al.*, 2005, 2006). Weight reduction due to moisture loss is more likely to occur in late-harvested tubers left in dry soil (Bridge, 1982). Water loss from tubers continues during storage and is significantly greater in tubers infected with *S. bradys* compared with healthy tubers (Adesiyani *et al.*, 1975b; Cadet and Quénéhervé, 1994).

Dry rot alone causes a marked reduction in the quality, marketable value and edible portions of tubers. When dry rot is followed by wet rot in stored yams, losses of whole tubers can be as high as 80–100% (Adesiyani *et al.*, 1975b), but losses certainly increase with duration of storage. The degree of preharvest damage to tubers by *S. bradys* varied from 0 to 40% in Nigeria (Wood *et al.*, 1980). During 3 months of storage, about 53% of yams with dry rot symptoms collected from West Africa had decayed completely and became unusable (Coyne *et al.*, 2005b). Also, almost 47% of all tubers on sale in Nigerian markets were infected with *S. bradys* (Bridge, 1973), and both dry rot and wet rot diseases of tubers have been observed in all Nigerian yam barns and markets sampled (Adesiyani and Odihirin, 1977). The proportion of infected tubers observed in markets across West Africa was lower, with the highest proportion of 7.5% infected tubers recorded from Ghana (Coyne *et al.*, 2005b). In Benin, 264 of 300 yam accessions collected during a countrywide survey in 2014–2015 were infected with *S. bradys* (A. Affokpon, unpublished data). Figures may fluctuate depending on the year, geography and time of the year of the survey. Nematode infection contributes to long-term storage losses, which have been estimated as 50% (Coursey, 1967).

Nematode densities in the outer peelings of rotted yam tubers can average 100,000 nematodes (Adesiyani *et al.*, 1975a) and can exceed 300,000 nematodes/50 g of tuber peelings (Bridge, 1973). Low population densities of the nematode produce only discrete areas of yellow necrotic tissues or dry rot internally, and densities in excess of 1000 nematodes/50 g of tuber peelings are necessary to produce observable external symptoms of damage (Bridge, 1973). Cadet and Daly (1996) found that nematicide treatment of seed infected with *S. bradys* gave 14–15% yield increase, but this was not significant.

Inoculation with 2000 *S. bradys* per mound resulted in no differences in yield compared with uninoculated plants, but during storage led to weight reductions of 40% after 3 months, compared with 28% for uninoculated tubers (Coyne *et al.*, 2012).

Management measures

Management options for *S. bradys* include: (i) treatment of field soil by chemical and cultural means; (ii) use of nematode-free or treated planting material; and (iii) treatment of tubers after harvest to prevent storage losses.

CULTURAL: keeping fallow land free of all host plants is a suggested control of *S. bradys*, but this is not always economical or practical.

The rotation of crops to control *S. bradys* is also not always an appropriate option as yams are often grown as the first crop in a rotation after fallow. However, with rising demands on land, fallow periods are reduced and cropping systems change, using poor or non-hosts in rotations, as intercrops or as cover crops will help reduce soil populations. The cover crops *Aeschynomene histrix*, *Centrosema pubescens*, *Pueraria phaseoloides*, *Mucuna pruriens* (*utilis*), *Stylosanthes guianensis* and *Tagetes erecta* are poor hosts, while *Cajanus cajan* is considered a useful trap crop for *S. bradys* (Carmo *et al.*, 2014; Claudius-Cole *et al.*, 2014). In Brazil, *Crotalaria spectabilis* Roth. and *Phaseolus lunatus* cv. Branca were recommended for controlling combined infestations of *S. bradys* and *P. coffeae* (Silva *et al.*, 2014). Some of these crops (*Tagetes* sp., *Stylosanthes gracilis*, *Centrosema* sp., *Aspilia latifolia*) and groundnut (peanut) have been recommended for lowering nematode densities and restoring soil fertility for yam production (Atu and Ogbuji, 1986). Mulching has also been reported to reduce nematode densities generally compared with pre-planting levels in the soil (IITA, 1976). Crops that are known to support high densities of *S. bradys*, such as cowpea, sesame, green gram, kenaf, okra, tomato, melon and potatoes should be avoided.

Yams are frequently intercropped, which may lead to build-up of nematode densities if the crops are hosts of *S. bradys*. For example, *S. bradys* significantly increased in yam intercropped with

cowpea, okra, tomato, cassava or sorghum (Adesiyon, 1976; Atu, 1991; Kwoseh and Krapa, 2008). Non-hosts of *S. bradys* should be used, where possible, to reduce damage to tubers. Intercropping yam with maize, ginger and cocoyam reduced soil nematode densities by 38%, 42% and 54%, compared with yam monocrop (Oluwatayo *et al.*, 2011). Similarly, weed control and the exclusion of weed hosts of *S. bradys*, such as *Eupatorium*, *Synedrella* and *Chromolaena*, from around yam will help to reduce nematode damage (Adesiyon, 1976).

The use of healthy, nematode-free seed material is by far the most appropriate means of preventing nematode damage. Seed tubers showing symptoms of dry rot (cracking and flaking), as can often be found on sale in local markets (Fig. 8.8), should not be used for planting. The presence of dry rot in tubers without external symptoms can be determined by scraping away sections of tuber skin to reveal the necrotic tissue beneath. Any foliar bulbils or aerial tubers used for planting will be completely free

of *S. bradys*. The generation of plantlets using *in vitro* tissue culture techniques, vine cuttings or aeroponic systems offers the potential to generate disease-free, healthy seed tubers and improve the international movement of germplasm (IITA, 2000; Coyne *et al.*, 2010a; Kabeya *et al.*, 2013; Maroya *et al.*, 2014; Dibi *et al.*, 2016). Even true seed can be used for propagating *D. rotundata* (Sadik and Okereke, 1975). Although these methods of propagation are not a practical means of producing ware tubers, they can be used to produce nematode-free seed tubers. The method used to produce large numbers of seed tubers from relatively few yams by growing 'microsetts' or 'minisetts' cut from mature tubers (Aighewi *et al.*, 2015; Dibi *et al.*, 2016) will effectively produce nematode-free propagative material as long as clean, healthy 'mother seed yams' are selected. The use of wood ash to coat yam setts before planting is a traditional practice among some yam growers and can enhance tuber formation, but is not suitable for managing nematodes (Kenyon *et al.*, 2005). Mixing



Fig. 8.8. Seed yam (*Dioscorea* spp.) tubers for sale at market in Ghana, exhibiting various levels of dry rot disease caused by *Scutellonema bradys*. (Photograph courtesy of D. Coyne.)

cow dung in yam mounds before planting at a rate of 1.5 kg/mound (1886 kg/ha) can increase yields of tubers and decrease nematodes significantly (Adesiyon and Adeniji, 1976). Other organic manures may also reduce nematode densities. The use of neem has also been investigated; nematode management has been observed and yields increased following the application of neem powder at 2.5 t/ha to the soil (Onalo *et al.*, 2001). The use of inorganic fertilizers has seen variable responses, with DAP and NPK suppressing nematode multiplication in tubers (Baimey *et al.*, 2006). In contrast, nitrogen application can increase *S. bradys* densities and the percentage of infected tubers of *D. rotundata*; phosphorus alone can decrease the percentage of infected tubers. Assessment of the effect of NPK, DAP and KCl on the densities and damage of *S. bradys* on yam in fields and in storage showed that weight loss was more pronounced in nematode-infected tubers from plots receiving fertilizers than from those receiving no fertilizer (Baimey *et al.*, 2006). However, the conditions under which experiments are conducted and the species of yam and cultivar used may influence the results obtained. For example, *S. bradys* densities increased on *D. rotundata* but not on *D. alata* or *D. cayenensis* following the application of high rates of nitrogen combined with phosphorus (Obigbesan and Adesiyon, 1981).

HOT WATER TREATMENT: hot water treatment (HWT), or thermotherapy, can reduce or eliminate *S. bradys* from tubers. While farmers appreciate the benefits of the treatment, the cost and the access to resources (e.g. firewood), the labour requirements, heating equipment and the difficulties of maintaining constant temperatures are the main prohibitive factors against its widespread use by farmers. However, it is feasible for small-scale operations and for establishing nematode-free planting material (Speijer, 1996; Coyne *et al.*, 2010b).

Most studies have shown that a water temperature of 50–55°C for up to 40 min gives the best control of *S. bradys* without damaging the tubers. The age of the tuber, the species of *Dioscorea* and the cultivar being treated, and the severity of infection of the tubers, will affect nematode control by HWT (Ayala and Acosta, 1971; Bridge, 1975; Acosta and Ayala, 1976b; Adesiyon and Adeniji, 1976; Castagnone-Sereino, 1988). However, HWT of tubers can affect

the sprouting ability of both cut setts and whole tubers adversely (Coyne *et al.*, 2010b). There is a high variability in the response of yam cultivars to HWT, with *D. alata* cultivars tending to sprout better than *D. rotundata* cultivars post-treatment. The time of treatment can be critical. *D. rotundata* tubers treated immediately after harvesting rotted completely, but those treated after a storage of 2–6 months showed little sign of deterioration, although those treated soon after dormancy had broken were slower to sprout (Bridge, 1975; Adesiyon and Adeniji, 1976). Coyne *et al.* (2010b) conclude that HWT appears potentially more detrimental than previously considered, but does enable the generation of clean stocks for use in generating healthy planting material.

RESISTANCE AND TOLERANCE: resistance to *S. bradys* in landraces or accessions of *D. alata*, *D. cayenensis* and *D. rotundata* remained an elusive goal over numerous screening assessments, with no firm evidence of complete resistance to *S. bradys* in the main food yams (*D. alata*, *D. bulbifera*, *D. cayenensis*, *D. esculenta* and *D. rotundata*) (Adesiyon, 1977; Bridge, 1982; Kwoseh *et al.*, 2002, 2007). However, variations in relative susceptibility have been reported, and *D. dumetorum* is generally considered to be invaded less readily than other species. *D. dumetorum* cv. Nkanfo and *D. cayenensis* cv. Afun supported low levels of *S. bradys* reproduction (Kwoseh *et al.*, 2007). The development of a high-throughput procedure, using vine cuttings, renewed the search for nematode resistance in line with renewed breeding efforts at IITA. Initially, one accession, TDr 98/00205, showed some promise in terms of reduced damage by *S. bradys* (Aremu, 2014); later breeding accessions, however, have begun to demonstrate encouraging levels of resistance in both *D. alata* and *D. rotundata*, offering real promise of resistance against *S. bradys* in yam (Odum, 2015; Kolombia, 2017).

CHEMICAL: chemical control of *S. bradys* on yams has had some success, with some remarkable yield increases recorded. Information on the economics of field chemical control, however, is mainly lacking for large-scale use, while concerns regarding residues in harvested tubers (Adesiyon and Badra, 1982) should be considered carefully and assessed when using for ware yam production.

There are numerous examples for the use of chemotherapy of tubers as a practical means of nematode control for yam growers, demonstrating significant increases in yield (Bridge *et al.*, 2005). Treatment of tubers would be more economical than treatment of the field, especially as *S. bradys* tends to be primarily tuber borne. However, the implications and consequences of residues in tubers pose some potential challenges. Ideally, treatment of tuber setts for the production of seed tubers would serve to overcome the residue issue. This was the premise behind a project to reassess healthy seed production systems in Nigeria, which evaluated various possible 'off-the-shelf' methods; the best identified method was a fungicide–insecticide (mancozeb and diazinon) combination dip or dust of seed setts (Kenyon *et al.*, 2005, 2006; McNamara and Morse, 2014). On average, yields of seed yam were doubled, which in turn led to multiple-fold increases in ware yam, based largely on *S. bradys* control. This was also due to reduced losses in storage from healthier tubers, as also observed after seed treatment with ethoprophos and cadusafos (Cadet and Daly, 1996). The danger however, is with farmers indiscriminately using pesticides on all yam planting material, which needs to be managed carefully; in West Africa, the availability, quality and understanding by farmers of pesticide use can be very variable. With numerous pesticides removed from the market due to their risks, and new products becoming available, the choice of pesticides should be made on current availability. Readily available household disinfectants and nitrogenous fertilizers, ammonium sulfate and calcium nitrate have also been assessed. Treatments reduce *S. bradys* in tuber tissues, but do not necessarily eliminate them (Badra and Caveness, 1979; Hutton, 1998). Soaking tubers in oxamyl prior to planting is recommended to control both *S. bradys* and *P. coffeae* (Castagnone-Sereno, 1988). A novel 'wrap and plant' method, using a banana fibre matrix as a carrier for abamectin (600 ng/0.3 m²), reduced *S. bradys* densities up to 86% and increased tuber yields by 22%, compared with the standard practice in Benin (Affokpon *et al.*, 2016a; Opperman *et al.*, 2016).

BIOLOGICAL CONTROL: controlling yam nematodes using biological control agents is gaining attention. Yam was identified as highly mycor-

rhizal in a study in Benin, and associated with a wide diversity of arbuscular mycorrhizal fungi (AMF) (Tchabi *et al.*, 2009). Pre-inoculation of micropropagated yam plantlets with AMF species *Funneliformis mosseae* and *Glomus dussii* led to significant nematode suppression in tuber, root and soil after 6 months (Tchabi *et al.*, 2010, 2016a). Preliminary results on the suppressive effect of *Trichoderma* species from West Africa on *S. bradys* are also optimistic (Kolombia, 2017).

INTEGRATED MANAGEMENT: there is the need to formulate an adaptable package that will suit the circumstances of the majority of yam farmers. However, a minimum and targeted use of chemical nematicides to lower high densities of nematodes in the soil, and management of these lowered densities with nematode-free planting material, is suggested. A critical component of any package, however, should be based on the use of healthy planting material that is made available and accessible to farmers through sustainable healthy seed delivery systems (Aighewi *et al.*, 2015).

Diagnosis

Assessment of the incidence and extent of dry rot disease in yam tubers can be made through direct observation. In tubers without obvious external symptoms of damage, it is necessary to scrape away the surface or to peel or section the tubers to assess for the presence of dry rot disease.

Nematodes will occur in soil and roots, which can be sampled, particularly at the end of the growing season. However, most nematodes occur in tuber tissues, and assessment of tuber tissue is most appropriate for evaluating the importance of *S. bradys*. Peel the tubers with a knife or kitchen peeler, chop finely, or preferably macerate, and extract using a modified Baermann method (see Chapter 4, this volume; Coyne *et al.*, 2014). In the first 3 days, 30–50% of nematodes will emerge from the tissues, but they will continue migrating from the tissues for over 20 days providing the water is replaced regularly/daily.

Pratylenchus coffeae

P. coffeae occurs throughout the tropics on numerous crops. It is recorded as a parasite of yams

in Central America, South America, China, Taiwan and in the Pacific Islands (Bridge *et al.*, 2005). Although *P. coffeae* occurs across Africa, it is not reported as a pest of yam in Africa, possibly due to variability in pathotypes or biotypes (Duncan *et al.*, 1999). Some reports have associated *P. coffeae* with yam in Ghana (Osei *et al.*, 2015) and Nigeria (Aremu, 2014; Kolombia, 2017), which require accurate characterization to help clarify the situation. *P. coffeae* is the cause of tuber dry rot disease of yams, known locally in Jamaica as 'burn'.

Symptoms of damage

The dry rot symptoms caused by *P. coffeae* in yam tubers are indistinguishable from those caused by *S. bradys*. Brown, irregular dry rot extends 1–2 cm into the outer tissues of *D. rotundata* tubers (Acosta, 1974), but can occur as deep as 5 cm in *D. alata* tubers (Bridge and Page, 1984). The dry rot can be more pronounced in the oldest apical portions of the tubers adjacent to the vines (Acosta, 1974), or even restricted to these portions in newly harvested tubers (Bridge and Page, 1984). External symptoms observed on tubers of *D. alata*, *D. cayenensis* and *D. rotundata* are deep cracks, a corky appearance, exposed dark brown rotted areas and diseased tubers being spongy to the touch (Thompson *et al.*, 1973; Acosta and Ayala, 1975; Bridge and Page, 1984). Necrosis or rotting caused by *P. coffeae* has also been observed in tubers of *D. esculenta* (Bridge and Page, 1984) and *D. trifida* (Hickling, 1974).

Above-ground symptoms of damage are not generally obvious. Vines from tubers severely infected with *P. coffeae* are shorter and unthrifty (Coates-Beckford *et al.*, 1978). Planting material with a high proportion of dry rot will reduce sprouting and result in poor stands in fields (Coates-Beckford and Brathwaite, 1977).

Biology

P. coffeae is a migratory endoparasite of yam roots and tubers. It is assumed to have a life cycle of 3–4 weeks on *Dioscorea* spp. (Thompson *et al.*, 1973), and the general behaviour of *P. coffeae* in yam tubers is probably similar to that of *S. bradys*.

No information is available on whether *P. coffeae* of yams is a separate biological race from those that are important parasites of other

crops, although the possibility exists (Duncan *et al.*, 1999). An isolate of *P. coffeae* from banana in Ghana multiplied in roots of yams, but tubers had few or no nematodes and no associated dry rot symptoms (Kwoseh, 2000).

P. coffeae reproduces and multiplies in stored yams and is disseminated in seed tubers. Densities increased from 185 nematodes/g tuber tissue at harvest to 1450/g at planting (Kermarrec *et al.*, 1988). HWT for 45 min at 45°C increased yields by 23% in Jamaica (Hutton *et al.*, 1982). Between yam crops, the nematodes can survive in field soil on other hosts. It can also be introduced into yam fields in the roots and plant tissues of other crop hosts. Contaminated machinery, tools, clothing and animals are an easy means of dispersal within and between fields (Adesiyan *et al.*, 1990).

Temperature can have a marked effect on nematodes. During storage, at ambient temperatures of 24–31°C, *P. coffeae* densities can rise to very high levels (939/g), but in tubers stored at 12–13°C, the densities remain low (<1/g) (Thompson *et al.*, 1973).

Other hosts

P. coffeae is a parasite of *D. alata*, *D. cayenensis*, *D. esculenta*, *D. rotundata* and *D. trifida*. It has also been associated with *D. bulbifera* in the Pacific Islands (Orton Williams, 1980). In addition to yam, *P. coffeae* has an enormous host range covering almost all plant families.

Disease complexes

Dry rot of yam caused by *P. coffeae* is associated with other soft and wet rots in stored tubers (Coates-Beckford and Brathwaite, 1977; Bridge and Page, 1984). It is likely that similar inter-relationships between nematodes and other organisms that have been described or suspected with *S. bradys* also occur with *P. coffeae*.

Economic importance

P. coffeae is important as a parasite of tubers, reducing their edible portions, marketable value and, particularly, their storage qualities. In Jamaica, 67–100% of *D. rotundata* and *D. cayenensis* tubers were infected with *P. coffeae* (Thompson *et al.*, 1973), and caused considerable losses to

the same yam species in Guadeloupe (Kermarrec *et al.*, 1988). Over 50% of *D. alata* tubers examined in Papua New Guinea had obvious signs of dry rot and were infected with *P. coffeae*, sometimes in excess of 60,000 nematodes/50 g (Bridge and Page, 1984). *P. coffeae* can cause 30–100% disease incidence on Chinese yam (Huang *et al.*, 1994).

P. coffeae primarily affects production as a result of using infected seed material, although when *P. coffeae* is present initially in the soil, yields can be affected through reduced-quality tubers. Soil densities of 600 *P. coffeae*/plant of *D. rotundata* can produce significant tuber damage, and 1000 nematodes/plant can cause complete deterioration and severe reduction in tuber quality. However, neither of these densities reduced the total weight of harvested tubers (Acosta and Ayala, 1975, 1976a). If seed tubers are badly affected, sprouting will not occur (Coates-Beckford and Brathwaite, 1977).

Management measures

The management options that have been described for *S. bradys* are, in most cases, applicable for *P. coffeae*. The main exception is in the use of crop rotations, because of the different host range of *P. coffeae*.

CULTURAL: using plant material that is free of nematodes is a key means of controlling or reducing damage by *P. coffeae*. As with *S. bradys*, central or distal tuber pieces, which generally contain the least *P. coffeae*, are recommended for propagative material (Acosta, 1974).

P. coffeae has an extremely wide and varied host range, and there are few reports of resistant crops against the yam isolates of *P. coffeae*, making it difficult to recommend any effective crop rotations. However, in Puerto Rico, rotating *D. alata* cvs. Kinabayo, Florido and Gunung with the highly susceptible *D. rotundata* cv. Habanero significantly reduced dry rot and improved the quality and yield of cv. Habanero (Oramas Nival and Rodriguez, 2002). The weeds *Rottboellia exaltata* and *Setaria barbata*, commonly found in yam plantations in Guadeloupe, are excellent hosts for *P. coffeae*, and it is recommended that they are removed (Kermarrec *et al.*, 1988). Soil treatment with chicken manure at 4 t/ha before planting reduced nematode densities by 53%

over untreated mounds in Brazil (Morais *et al.*, 2016). The use of cattle manure also significantly reduced the incidence of dry rot in Brazil (Santos *et al.*, 2009).

PHYSICAL: while not always practical, HWT can reduce tuber densities of *P. coffeae* markedly, but rarely eliminates them without damaging the tuber. HWT at 46–52°C for 15–30 min has been recommended for control of *P. coffeae* in *D. rotundata* tubers (Acosta and Ayala, 1976b) and at 51°C for 15–35 min in *D. rotundata* and *D. cayenensis* tubers (Coates-Beckford *et al.*, 1978; Kermarrec *et al.*, 1988). Treatment of tubers with extreme dry rot should be avoided, as the treatment will be less effective. HWT can cause severe physiological damage (Thompson *et al.*, 1973; Coates-Beckford and Brathwaite, 1977; Coyne *et al.*, 2010b).

RESISTANCE AND TOLERANCE: it is suggested that *D. alata* cv. Florido is not susceptible to attack by *P. coffeae* (or *S. bradys*) in Puerto Rico (Ayala and Acosta, 1971). *D. esculenta* is possibly less susceptible to *P. coffeae* because of its different growth habit (Bridge and Page, 1984). Of 36 *D. rotundata* accessions screened in Nigeria, none were found resistant to *P. coffeae* using vine cuttings (Aremu, 2014).

CHEMICAL: as with *S. bradys*, the economics of chemical field treatments for *P. coffeae* have not been determined, while a number of chemicals evaluated have since been removed from the market, such as aldicarb and carbofuran.

Chemical treatments of tubers prior to planting or storage can greatly reduce *P. coffeae* in tubers, such as oxamyl dips (Oramas Nival, 2002), but no treatment appears to eliminate nematodes from tubers completely. Significant increases in yield of *D. rotundata* have also been obtained by a combination of foliar and seed tuber treatments with oxamyl (Roman *et al.*, 1984). In Jamaica, readily available household disinfectants, 'Dettol' or 'Jeyes Fluid', and bleach were as effective as oxamyl in controlling *P. coffeae* and dry rot on tubers dipped prior to planting (Hutton, 1998).

Meloidogyne species

The root knot nematodes *Meloidogyne* spp. infect yams wherever they are grown (Bridge

et al., 2005). Globally, *M. incognita* appears the most widespread and important, while *M. arenaria*, *M. enterolobii*, *M. hapla* and *M. javanica* are also reported from yam. *M. incognita* was the most prevalent species on yam in Ghana (Kwoseh, 2000) and Nigeria (Kolombia, 2017), and *M. javanica* in Benin (Affokpon *et al.*, 2016b). *M. enterolobii* was reported for the first time in Nigeria, causing severe galling damage on yam (Kolombia *et al.*, 2016), but is mostly present in mixed populations with *M. incognita* and/or *M. javanica* (Kolombia, 2017).

Symptoms of damage

Meloidogyne spp. cause typical knotting or galling of yam roots. More visibly striking, however, are the abnormal warty or knobby tubers that result from infection (Fig. 8.9). In cross section, females can be seen embedded in the discoloured tissue below the crenulated surface (Fig. 8.10). In older tubers, the tissue can become darkened and necrotic, surrounding individual females. Internal rotting, associated with *Meloidogyne* spp., can also occur in certain yam species. Galled tubers also give rise to a proliferation of roots, referred to as 'crazy root symptoms' (Fig. 8.11). Sprouting of galled tubers can be reduced or delayed (Schieber, 1961; Jenkins and Bird, 1962; Bridge, 1973; Kermarrec, 1974; Adesiyun and Odihirin, 1978; Nwauzor and Fawole, 1981; Mudioppe *et al.*, 2012). Minisets cut from heavily infected tubers senesced early and produced galled yam tubers that rotted completely in storage (Onyenobi *et al.*, 2006). *Meloidogyne* spp.



Fig. 8.9. Deformed warty yam (*Dioscorea rotundata*) tuber caused by *Meloidogyne* spp. (Photograph courtesy of D. Coyne.)

infection of tubers exacerbates tuber weight loss during storage, with more severely galled tubers losing more than double the weight of non-galled tubers (Mudioppe *et al.*, 2012).

Foliar symptoms on food yams are observed occasionally. Early yellowing, leaf fall and termination



Fig. 8.10. Cross section of *Meloidogyne* spp. infected crenulated yam (*Dioscorea rotundata*) tuber showing females embedded in the discoloured tissue. (Photograph courtesy of D. Coyne.)



Fig. 8.11. 'Crazy root' syndrome of yam (*Dioscorea rotundata*) tuber caused by *Meloidogyne* spp. infection. (Photograph courtesy of Y. Kolombia.)

of vine growth have been seen on *D. rotundata* infected with *M. incognita*, but infection only rarely reduces the total tuber yield of these yams (Adesiyan and Odihirin, 1978; Nwauzor and Fawole, 1981; Atu *et al.*, 1983). *M. incognita* produces obvious galling on tubers of *D. trifida* (Kermarrec, 1974) and on *D. rotundata*, *D. alata* and *D. praehensilis*, as well as intraspecific yam hybrids (Kwoseh, 2000). Seedlings of 'medicinal' yams (*Dioscorea composita*, *Dioscorea floribunda* and *Dioscorea spiculiflora*) can be severely stunted or killed by *M. arenaria* and *M. incognita*, mainly the latter, with foliar chlorosis and leaf dieback (Schieber and Lassman, 1961; Jenkins and Bird, 1962; Bruhn and Koch, 1963).

Biology and life cycle

The behaviour of *Meloidogyne* in yam roots is similar to that in other crops, but in tubers there are some unusual features. The life cycle of *M. incognita* in *D. rotundata* or *D. alata* tubers is 35 days (Nwauzor and Fawole, 1981). In *D. alata*, most nematodes are concentrated to a depth of 2 mm, with none beyond 8 mm depth; in *D. rotundata*, they are concentrated at depths between 4 and 6 mm, with few at 14 mm (Nwauzor and Fawole, 1981). Females and egg masses produced in tuber tissues of *D. composita*, *D. floribunda* and *D. spiculiflora* become surrounded by lignified cells, preventing the migration of hatched juveniles into surrounding tissues and causing their death (Bruhn and Koch, 1962; Jenkins and Bird, 1962; Koch, 1975). In *D. rotundata*, a similar host reaction was observed with *M. incognita*, which either kills or decreases juvenile and egg populations in stored tubers (Bridge, 1973; Nwauzor and Fawole, 1981). Mudiope *et al.* (2012) recovered juveniles from tubers up to 4 months after harvest. However, there was no evidence of reinfection of the tubers by juveniles and their further development into females. *M. hapla* develops in tubers of *Dioscorea batatas* (= *D. opposita*) until eggs are produced, and these hatch only when the tuber decays (Kawamura and Hirano, 1961).

Survival and dissemination

Where *Meloidogyne* juveniles or eggs survive in stored tubers, they will be disseminated in propagative material. However, damaging populations

may readily survive on weed hosts, or be introduced into yam fields on the infected seedlings of other crops.

Hosts

Susceptible yam hosts of *M. incognita* are *D. alata*, *D. bulbifera*, *D. cayenensis*, *D. composita*, *D. esculenta*, *D. floribunda*, *D. praehensilis*, *D. rotundata*, *D. spiculiflora* and *D. trifida*; hosts of *M. javanica* are *D. alata*, *D. cayenensis*, *D. opposita* and *D. rotundata*; *D. opposita* (= *D. batatas*) is a host of *M. arenaria* in China (Gao *et al.*, 2000) and *M. hapla* in Japan (Kawamura and Hirano, 1961) and Korea (Park *et al.*, 1998). In addition to yam, *Meloidogyne* spp. have very broad host ranges on weeds and crop plants.

Disease complexes

Yam tubers infected with *Meloidogyne* spp. are more prone to fungal and/or bacterial rot during storage than tubers free of the nematodes (Schieber, 1961; Schieber and Lassmann, 1961; Badra *et al.*, 1980; Nwauzor and Fawole, 1981).

Economic importance

Meloidogyne spp. adversely affect the marketable value of tubers because of the unappealing warty appearance. They are also associated with rot and more rapid loss of weight during storage.

Various studies assessing the effects of *Meloidogyne* spp. on yam demonstrate their negative impact on production and quality, but are variable and sometimes contradictory. *M. incognita* completely destroyed a crop of *D. trifida* in Martinique at soil densities of 30,000 juveniles/100 g (Kermarrec, 1974), and in Nigeria a combination of root knot and *S. bradys* caused the abandonment of large areas of yam farms (Adesiyan and Odihirin, 1977). Yields of yams severely infected with *M. arenaria* were reduced by 24–80% in China (Gao, 1992). *M. javanica* densities of 30,000/plant reduced yields of *D. opposita* by over 50% (Nishizawa, 1973). Lower densities (5000/plant) of both *M. incognita* and *M. javanica* significantly reduced yields of *D. alata*, but not of *D. cayenensis* or *D. rotundata* (Adesiyan and Odihirin, 1978). Densities as low as 100 *M. incognita* juveniles/plant can reduce tuber yields of *D. rotundata* in India (Mohandas and

Ramakrishnan, 1997). In Uganda, inoculation with 3000 eggs reduced *D. rotundata* tuber yields by 30%, while *D. alata* was unaffected (Mudioppe *et al.*, 2012). Various studies have shown that *M. incognita* and *M. javanica* do not appreciably decrease tuber weights however (Acosta and Ayala, 1975; Nwauzor and Fawole, 1981; Atu *et al.*, 1983; Atu and Ogbuji, 1986).

The tuber quality is of primary importance in determining the economic damage caused by root knot nematodes. Unsightly tubers are less appealing, but importantly have less edible portion (more peel has to be removed) and the flesh becomes corky and tough. It is estimated that there is a reduction of 39–52% in the price of galled tubers compared with healthy ones (Nwauzor and Fawole, 1981). The proportion of yams with galled tubers collected from yam barns and markets in Nigeria can be as high as 90% for *D. alata* and 70% for *D. rotundata* (Adesiyun and Odihirin, 1978). Proportions of galled tubers ranged up to 59% from market stalls and up to 27% in yam barns in Nigeria (Kolombia, 2017), which was higher than previously encountered (Coyne *et al.*, 2005b). Figures may fluctuate depending on the year, geography and time of the year of the survey, but in Nigeria it is generally recognized that root knot damage is becoming increasingly problematic on yam

(Kolombia, 2017) (Fig. 8.12). In Nigeria, the economic threshold at which control measures should be initiated is suggested when 40% or more of tubers are galled. This is based on differences in market value between infected and healthy tubers. Experimentally, this has been shown to occur when soil populations of *M. incognita* at planting are 50–250 eggs/plant (Atu *et al.*, 1983).

Management measures

There are a few specific control measures that can be used against root knot nematodes, but in general many of those described above for other yam nematodes can be applied.

CULTURAL: the carry-over of high densities of root knot nematodes in seed tubers has not traditionally been viewed as serious a problem as it is with dry rot nematodes (Nwauzor and Fawole, 1981). This is probably due to the fact that low densities of dry rot nematodes are visibly asymptomatic and will go undetected, while root knot nematodes create visible galling damage. The use of obviously galled tubers for propagative material should be avoided and awareness raised among farmers to this effect. This is important where, due to the low market prices, galled



Fig. 8.12. Heaps of yam (*Dioscorea rotundata*) tubers for sale at market in Nigeria, exhibiting high and severe levels of galling caused by *Meloidogyne* spp. infection. (Photograph courtesy of Y. Kolombia.)

tubers are held back from the market and may be used for planting material when times are hard (Nwauzor and Fawole, 1981). In Ghana, farmers remarked that knotted yam tubers were only observed on the fourth consecutive crop (Kwoseh, 2000). Continuous cropping of yam on the same piece of land should be avoided.

Crop rotation will be difficult for *Meloidogyne* spp. management because of their wide host range, but crops highly susceptible to root knot nematodes should be excluded from a cropping system. In Nigeria, intercropping highly susceptible crops such as cowpea, okra, lablab bean, pigeonpea, pumpkin, West African sorrel (*Corchorus olitoris*) and African yam bean (*Sphenostylis stenocarpa*) with yam increases tuber damage by *M. incognita* (Atu and Ogbuji, 1986; Adegbite *et al.*, 2005; Claudius-Cole *et al.*, 2014). Cover crops such as *C. pubescens*, *C. juncea*, *Mucuna* spp., *P. phaseoloides* and *S. gracilis* intercropped with yam led to reduced tuber damage due to *M. incognita* (Adegbite *et al.*, 2005; Claudius-Cole *et al.*, 2014).

PHYSICAL: HWT can be successful in controlling *Meloidogyne* spp. in tubers. As before, the economics and the success of the method will depend on many factors, including species and age of yam tubers, nematode densities and depth of infection. Immersing *D. alata*, *D. rotundata* and *D. floribunda* tubers at 50–51°C for 30 min can effectively eliminate *Meloidogyne* (mainly *M. incognita*) from galled tubers (Hawley, 1956; Nwauzor and Fawole, 1981), but is not entirely practical for smallholder farmers.

RESISTANCE AND TOLERANCE: the only yam species consistently demonstrating resistance to *M. incognita* is the cluster yam, *D. dumetorum* (Nwauzor and Fawole, 1981; Atu *et al.*, 1984; Kwoseh, 2000). However, given the rising number of species recorded on yam, the known geographic variability within *Meloidogyne* species and the multiple species infection of individual plants/tubers, local screening is strongly advised, even for cultivars designated as resistant against a particular species elsewhere. In various screening studies, there appears to be greater susceptibility to *Meloidogyne* species than resistance (Bridge *et al.*, 2005). *D. alata* cv. Obunenyi is reported to be resistant to *M. incognita* in Nigeria (Atu *et al.*, 1984), and *D. cayenensis* can be resistant

to *M. incognita* and *M. javanica* (Adesiyun and Odihirin, 1978; Nwauzor and Fawole, 1981). *D. esculenta* cv. Sree Latha is resistant to *M. incognita* in India (Mohandas *et al.*, 1996). One accession, TDr 98/00205, of 36 *D. rotundata* accessions screened, was found to be resistant to *Meloidogyne* spp. using vine cuttings (Aremu, 2014), while 67 *D. rotundata* and 18 *D. alata* accessions were found to be resistant in newly bred lines using the same screening system (Odum, 2015).

Two yam lines planted in a field naturally infested with *M. javanica* in Uganda were uninfected at harvest, while most lines were heavily infected, indicating resistance in these lines (Mudioppe *et al.*, 1998). Lowe (1992) also reported that other lines of *D. rotundata* were not attacked by *M. incognita* race 2 and *M. javanica*.

CHEMICAL: in Nigeria, a number of studies report on the control of root knot nematodes using carbofuran (see Bridge *et al.*, 2005), which has now officially been withdrawn from the market. Granular oxamyl at rates of 3 or 6 kg a.i./ha applied at planting and at three 4-week intervals can control *M. javanica* on *D. rotundata*. In the presence of both *M. javanica* and *P. brachyurus*, tuber yields can be increased by over 40% when granular oxamyl at 3 kg a.i./ha applied at planting is combined with subsequent repeated applications of calcium nitrate or ammonium sulfate. These treatments also reduce the incidence of rot in stored yams associated with the nematodes (Badra *et al.*, 1980).

BIOLOGICAL CONTROL: there is limited information regarding the use of biological agents for the control of root knot nematodes on yam. In pots in Benin, the assessment of two commercial AMF products, based on *F. mosseae* and *G. dussii*, on yam growth and root knot nematodes showed, in some cases, significant suppression of galling symptoms and higher tuber yields of *D. alata* and *D. rotundata* (Tchabi *et al.*, 2016b).

INTEGRATED MANAGEMENT: yam farmers traditionally intercrop yam with other crops. Consequently, the host status of these and other crops in the rotation to the nematode species present would be useful in selecting those most suitable in lowering field densities. Adaptation of strategies to disinfect seed material, such as HWT, and creating healthy seed systems, especially

based on resistant material, would benefit the production of yam through improved nematode management. However, it is also crucial that farmer awareness on understanding nematodes, nematode problems and how to manage them is facilitated towards them appreciating the benefits of implementing nematode management options.

Pratylenchus sudanensis

P. sudanensis was first observed on yam in 1993 during field studies in Uganda (Coyne *et al.*, 2003), where it was present at mean densities of 468/10 g root + 100 ml soil and since reported as the dominant nematode species occurring on yam in Uganda, at up to 1500 nematodes/g tuber (Mudiope *et al.*, 2007). Although yam is not a key staple crop in Uganda, it is locally important and gradually increasing in popularity (Coyne *et al.*, 2005c). The nematode is associated with cracked tubers (Mudiope *et al.*, 2004, 2008), which correlates strongly with nematode densities (Mudiope *et al.*, 2007). Cracking of tubers is also associated with an undefined condition, which results in rapid tuber deterioration (N. Wanyera, Uganda, 2002, personal communication). Inoculation with 100 and 10,000 *P. sudanensis*/plant in pots resulted in 28% and 52% dead roots, compared with 3% in uninoculated plants (Mudiope *et al.*, 2004). Host range studies in Sudan showed a number of favourable hosts, especially cotton (cvs. Barakat and Barakat(67)B), sorghum (cv. Dwarf White Milo), pigeonpea (cv. Local) and lubia bean (cv. Local) (Saadabi, 1985). Wheat (cv. Giza 155) and groundnut (cv. Ashford) were considered poor or non-hosts. *P. sudanensis* is morphologically similar to *P. pseudopratensis*, creating some difficulty in differentiating between the two species (D.J. Hunt, UK, 2003, personal communication); a morphological continuum may exist between the two species, which may be better delineated using molecular analysis.

Other nematode parasites of yams

Other species of *Pratylenchus* are known to be parasites of yam. *P. brachyurus* has been found in

tubers, roots and yam soil in West Africa (Caveness, 1967b; Kolombia, 2017), Guatemala (Jenkins and Bird, 1962), Fiji and Tonga (Bridge, 1988) and Brazil (Andrade *et al.*, 2010). *Pratylenchus scribneri* was recovered from one tuber sample from Nigeria (Kolombia, 2017).

Radopholus similis causes a dry rot of yam tubers in Papua New Guinea (Bridge and Page, 1984) and in New Caledonia. The disease is similar to that caused by *P. coffeae* and *S. bradys*, but diseased tissues tend to be lighter brown in colour (Bridge and Page, 1984). *R. similis* also infects tubers in Fiji (Butler and Vilsoni, 1975) and yam roots in the Solomon Islands (Bridge, 1988).

Aphelenchoides besseyi, a foliar nematode, is known to occur in high densities in the foliage and tubers of *D. trifida* in Guadeloupe, associated with drying and blackening of the foliage, and wasting and cracking of tubers with internal decay (Kermarrec and Anais, 1973).

A 'black scurf-like syndrome' of Chinese yam, *D. opposita*, was shown to be caused by *Paratrichodorus porosus* in Japan (Nishizawa, 1973). Symptoms of the disease are blackening, cracking and corkiness of the tuber tips. The disease increases in severity with successive planting of yams. *P. porosus* reduces tuber weight and greatly inhibits their elongation, resulting in small, rounded, rather than long, thin tubers.

Of the remaining nematodes associated with yams, the only other species identified as parasites of yam roots or tubers are *R. reniformis*, *S. clathricaudatum* and *H. dihystra*, although neither *S. cavenessi* nor *S. clathricaudatum*, both known to occur in West Africa, were found infecting stored yam tubers in Ghana (Kwoseh, 2000).

Taro

Taro (*C. esculenta*), also known as cocoyam, dasheen and eddoe, is grown throughout the tropics, subtropics and warmer regions of the temperate zone. It belongs to the Araceae family and is believed to have originated in South-east Asia. It is mostly a staple food or subsistence crop, but is grown commercially in some countries. There are two botanical varieties of *Colocasia*, the 'eddoe type' *C. esculenta* var. *antiquorum*, which has a relatively small corm surrounded by large, well-developed cormels, and the 'dasheen type' *C. esculenta* var. *esculenta*, which has a large

central corm and numerous but small cormels. They can be grown in dry upland or flooded areas, depending on the type and cultivar.

Taros are propagated vegetatively using whole corms or cormels, pieces of corms or the leaf-bearing tops of mature corms (the lower 30–50 cm of the petiole with the top 1–2 cm of the corms).

Nematodes of Taro

The nematodes known to be damaging parasites of taro are *Meloidogyne* spp., *Hirschmanniella miticausa* and *P. coffeae*. Other nematodes found associated with tissue damage or present in high densities on the crop are *Radopholus* sp. and *R. reniformis*.

Meloidogyne species

The root knot nematodes, *Meloidogyne* spp. (*M. incognita*, *M. javanica* and *M. arenaria*), are the most widespread reported nematodes on *Colocasia*, from Central America, Hawaii, Africa, the South Pacific, the Philippines, Taiwan, Japan and India; and *M. hapla* additionally recorded from Uganda (see Bridge *et al.*, 2005).

Symptoms of damage

Both *M. incognita* and *M. javanica* can cause galling of roots and corms. Galls are small and irregular on young feeder roots. Infected older roots become thickened with large swellings, although the symptoms are not always obvious, as in Tanzania, where no external galls were visible on infected roots, even at densities of up to 6570 juveniles/g root (Runkulatile and Teri, 1990). On corms, nematodes cause blister-like swellings, which later become large round or oblong galls, 2–15 mm in diameter, deforming the corms, which rot in storage. Nematodes may create yellow-coloured areas of variable size within corms, even though external symptoms are not present. Above-ground symptoms occur in patches of stunted and unhealthy plants with yellowed leaves, which can turn brown and die (Nirula, 1959; Srivastava *et al.*, 1971; Brathwaite, 1972; Lorenzo and Fernandez, 1982).

Survival and means of dissemination

The use of *Meloidogyne* spp. infected corms and cormels for planting poses the greatest mode of transmission to new crops. Transfer can also occur from one crop to the next on other host crops and weeds.

Environmental factors affecting parasitism

Root knot nematodes are especially serious on the eddoo type or upland taro, *C. esculenta* var. *antiquorum*; *Meloidogyne* can be suppressed when taro is grown in very wet or flooded conditions (McSorley *et al.*, 1983a).

Economic importance

Losses caused by *Meloidogyne* have been described as severe in India, where local farmers have abandoned cultivation of *Colocasia* because of the nematodes (Srivastava *et al.*, 1969). It is suggested that *Colocasia* (and *Xanthosoma*) are more tolerant of *M. incognita* than other crops, and that high pre-plant densities are required for damage to occur (McSorley *et al.*, 1983a). The malformation of corms due to galling reduces their marketable value (Srivastava *et al.*, 1971). The yield of the susceptible cultivar Sree Pallavi was reduced significantly by 21% following inoculation with 1000 *M. incognita* juveniles in pots in India (Mohandas and Palaniswami, 1991). Although no external galls were observable, high nematode densities resulted in severe damage to cocoyams in Tanzania (Runkulatile and Teri, 1990).

Management measures

The use of nematode-free planting material will prevent dissemination into the field. However, as external symptoms are not always expressed, seed corms or cormels should be selected from land with no previous nematode problem. The treatment of planting material will provide greater surety of preventing contamination, however. Root knot can be controlled in corms using HWT, but this may be uneconomical. For importation of corms into New Zealand, HWT has been evaluated as a replacement of methyl bromide; 100% mortality of juveniles was achieved in 1–2 min at 47.5°C, and 30 s to 1 min at 50°C and for 45 s at 52.5°C (Jamieson *et al.*, 2016).

Cover crops can be an effective and valuable nematode management tactic for use in minor tropical cropping systems, such as taro (Sipes and Arakaki, 1997). Based on field trials, marigold cultivars, sorghum–sudangrass hybrids and sunn hemp were the best rotation green manure crops for reducing *M. javanica* in an upland taro cropping system (Miyasaka *et al.*, 2016).

The number of contradictory reports on damage by *Meloidogyne* may be due to the different host reactions of the many taro cultivars grown worldwide (McSorley *et al.*, 1983a). One cultivar, 'Dodare', in Japan was completely resistant to both *M. incognita* and *M. javanica* (Inagaki, 1981), while cv. 'Samra' in Fiji was described as moderately susceptible to these two species (Kirby, 1977). In India, cv. C9 is classed as immune to *M. incognita* (Mohandas *et al.*, 1996). The cvs. Mana Ulaulu and Piko Ulaulu are possible sources of partial resistance and/or tolerance to *M. javanica* in Hawaii (Sipes *et al.*, 1995). Various taro germplasm from Thailand, Vietnam and Nepal and 11 hybrids all supported *M. javanica* reproduction in the greenhouse, although one accession from Thailand and two hybrids showed some resistance (Ortiz *et al.*, 2008).

Diagnosis

Standard methods for the extraction of nematodes from soil and roots can be used (see Chapter 4, this volume). Assessing for *Meloidogyne* in corms and the damage they cause can be conducted in a similar way to that used for yam tubers.

Hirschmanniella miticausa

H. miticausa is the causal organism of 'miti-miti', a taro corm rot disease. The disease and nematode are reported from four islands in the Solomon Islands group (Mortimer *et al.*, 1981) and the highlands of Papua New Guinea (Bridge and Page, 1984), while a *Hirschmanniella* sp. has been recorded associated with taro in Taiwan (Huang *et al.*, 1972).

Symptoms of damage

The initial foliar symptoms of miti-miti disease are wilting of the older leaves, which eventually become chlorotic, while the new central

leaf, instead of bending, remains straight. Infected plants die prematurely as a result of corm damage.

Infected corms, when cut, initially show red streaks radiating from the base of the corm, which develop into irregular, 1–10 mm wide, zones of dry brown rot, with the advancing diseased tissues remaining red (Fig. 8.13). The basal portions of severely diseased corms are often completely decayed, due to a brown soft rot. Fewer cormels are produced on diseased plants (Mortimer *et al.*, 1981; Bridge *et al.*, 1983).

Biology

H. miticausa is a migratory endoparasite occurring mostly in the corms, less in roots and relatively few in the surrounding soil. Nematodes are found in, or immediately around, red necrotic tissues of the corm in the basal portion. They are relatively scarce in the white central tissues and rare in the crown (top 1 cm). Densities can commonly exceed 1000/10 g and can be over 3000/10 g corm tissue. The nematode is disseminated in infected taro corm planting material; no other hosts are known.

Disease complexes

Nematode activity in corm tissues probably predisposes the corms to invasion of secondary pathogens, causing the extensive outer soft rot invariably associated with the disease. Fungi isolated from areas of soft rot in corms with miti-miti are *Corticium solani*, *Pythium vexans*, *Fusarium solani* and *Fusarium oxysporum* (Bridge *et al.*, 1983).

Economic importance

Miti-miti disease renders taro corms inedible and, when severe, destroys almost all consumable corm tissues. Taro cultivation was almost entirely abandoned in parts of the Solomon Islands because the disease was so devastating (Patel *et al.*, 1984).

Management measures

Planting material infected with *H. miticausa* is the main source of inoculum for new fields. Nematodes can be eliminated from normal planting material (corm top and 40 cm of leaf

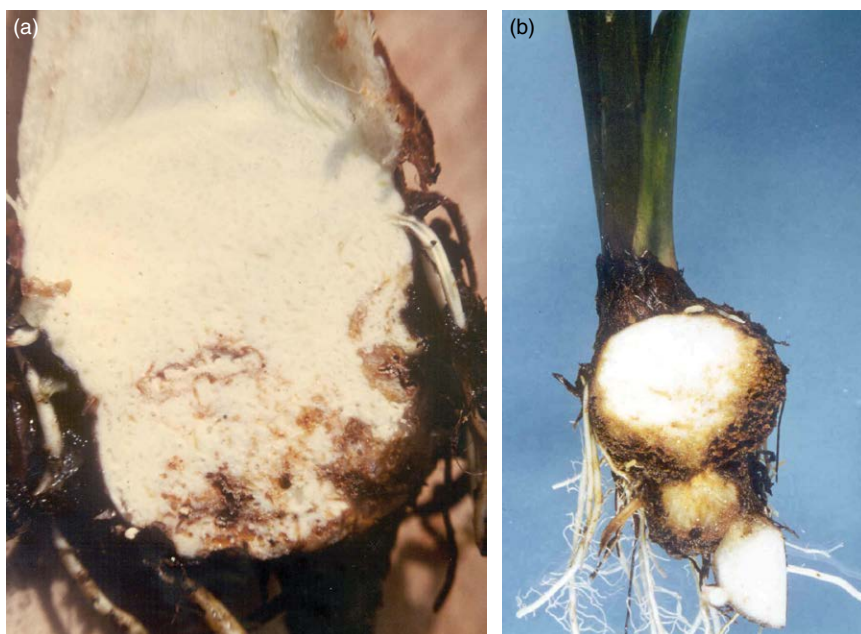


Fig. 8.13. Early stages showing reddening of tissues (a) and more advanced diseased condition (b) of miti-miti disease caused by *Hirschmanniella miticausa* in taro corm in longitudinal section. (Photograph courtesy of J. Bridge.)

base) by HWT at 50°C for 15 min without damaging the tissues (Mortimer *et al.*, 1981). Due to the difficulties of treatment, it cannot be generally recommended to taro growers, but it could be used to establish a source of nematode-free planting material.

The most practical measure for small growers is to trim the corm top manually back to white, healthy tissues, as nematodes rarely occur in the top few centimetres of the corm. This should ensure that most, if not all, planting material is free of nematodes (Mortimer *et al.*, 1981).

Where taro is grown on hillsides, there is a risk of nematodes being carried downhill in runoff water. This can be avoided by making new plantings uphill from old taro gardens (Mortimer *et al.*, 1981).

These hygiene measures cannot be used in areas where there is intensive and continuous taro production, such as in swamp pits. A practical solution would be the use of resistant cultivars. A local unspecified wild accession has been identified in the Solomon Islands as resistant to the nematode, but it is low yielding and acrid

when cooked. There is potential for crosses between this taro and high-yielding cultivars though (Patel *et al.*, 1984).

Diagnosis

H. miticausa is a large nematode and is extracted from soil most efficiently by a sieving and sedimentation method (see Chapter 4, this volume). However, as most nematodes are found in plant tissues, their extraction from corms will give the most accurate assessment of their presence and population levels using a standard tissue extraction method.

Pratylenchus coffeae

While the lesion nematode *P. coffeae* has been recorded on taro in various parts of the Pacific (Bridge *et al.*, 2005) it has been reported causing injury to taro only in Japan (Nishizawa and Ohshima, 1972; Oashi, 1984; Inagaki, 1985; Yamada, 2001), where it occurs in up to 45% of fields (Iwahori *et al.*, 2000). Due to

microevolution in the *P. coffeae* species complex, however, the specific species involved may be more complex (Mizukubo *et al.*, 2003); molecular assessment further indicates that certain *P. coffeae* populations from *C. esculenta* in Japan need to be considered as a new species (De Luca *et al.*, 2012).

Symptoms of damage

P. coffeae has been consistently associated with a disease of taro in Japan causing poor plant growth, root decay and a reduced number of corms. Two months after planting, roots turn brown and then rot. This is followed by stunted top growth and, in serious cases, withering and death of the leaves 5 months after planting. The disease is most common in fields with continuous taro cultivation (Oashi, 1984). In Papua New Guinea, *P. coffeae* causes localized necrosis of root and corm tissues (Bridge and Page, 1984).

Biology

All stages of *P. coffeae* are found in roots, corms and in the soil around taro. Highest densities occur in roots and soil, with less in the 'skin' of the corms (Oashi, 1984).

Economic importance

Field trials have shown that, by controlling *P. coffeae* in seed corms and field soil, corm yields can be increased threefold. The most serious damage and highest nematode densities occur where taro is cultivated continuously, although nematodes may not be the only cause of problems with continuous taro cultivation (Oashi, 1984).

Management measures

Management measures for *P. coffeae* on taro include using healthy seed corms, the reduction of soil populations and crop rotation (Oashi, 1984).

Seed corms selected from healthy parent plants are recommended, and that all roots are removed before planting. In Japan, nematodes can be eliminated from corms by soaking in a disinfectant ('cartap aqueous solution') for 30 min (Iwahashi, 1977), but chemical residues may be a problem. HWT could also feasibly be effective (Jamieson *et al.*, 2016).

The lowest densities of *P. coffeae* are found in soils that have previously been flooded, and planting taro in rice paddy field soil compared with dry, upland soil reduces the risk of damage. Combining disinfection of seed corms with cultivation in paddy soil can almost eliminate nematodes.

Although nematicides will provide some control of *P. coffeae*, it is viewed as uneconomical (Torigoe, 1993). Crop rotation is considered a more appropriate control measure. Soil densities of *P. coffeae* are reduced following groundnut, marigold, radish and *Stevia rabaudiana* but the nematodes increase to large densities as soon as taro is cultivated, and a rotation of 2 or more years between taro crops is necessary (Oashi, 1984; Torigoe, 1994; Yamada, 2001). The use of antagonistic plants such as *C. juncea* and *C. spectabilis* in rotation can be effective against *Pratylenchus* (and *Meloidogyne*) (Torigoe, 1996). The cultivation of palisade grass (*Brachiaria brizantha*) cv. MG5 has a suppressive effect on *P. coffeae* that is comparable to fallow treatments (Uesugi *et al.*, 2015).

Diagnosis

Determining the presence of nematodes in association with diseased plants will require sampling and extraction from soil and plant tissues. It will not always be possible to obtain a direct association between visible root damage and nematode densities as *P. coffeae* can be found in superficially healthy white roots (Oashi, 1984).

Other nematodes of taro

R. reniformis has been recorded from *Colocasia* in Puerto Rico, Taiwan, Fiji, Western Samoa, the Solomon Islands, Tonga, India, Japan and Florida (Bridge *et al.*, 2005). Although high levels of *R. reniformis* (1767 nematodes/100 cm³ soil) could be found with *Colocasia*, no effect on yield was noted in Florida (McSorley *et al.*, 1983a).

An undescribed *Radopholus* sp. is reported from necrotic tissues of taro corms and roots in Papua New Guinea (Bridge and Page, 1984). *Radopholus* spp. have been associated with taro in Fiji, Tonga and Western Samoa (Kirby *et al.*, 1980; Orton Williams, 1980).

A. besseyi is recorded in large densities from taro corms with rot (Bridge and Page, 1984).

Xanthosoma

There are about 40 species of *Xanthosoma* with the common names of tannia, tanier, yautia, malanga and new cocoyam. They can be confused with the genus *Colocasia* because of their similar botany, but are distinguished by their different leaves. The most widely grown edible species is *Xanthosoma sagittifolium*; others are *Xanthosoma atrovirens*, *Xanthosoma violaceum*, *Xanthosoma caracu* and *Xanthosoma brasiliense*.

Tannias are propagated vegetatively from pieces of main corm, cormels, or the tops of the main corm plus 20–30 cm of leaves. They cannot withstand waterlogging, and prefer an average annual rainfall of 140–200 cm (Purseglove, 1972; Kay, 1987).

Nematodes of *Xanthosoma*

Comparatively little information is available on the importance of nematodes associated with *Xanthosoma*. Only *Meloidogyne* spp., *R. reniformis* and *P. coffeae* are reported to cause damage to the crop.

***Meloidogyne* species**

Four species of *Meloidogyne* are reported from *Xanthosoma*: *M. hapla* from Uganda, *M. arenaria* from Cuba and Tanzania; *M. incognita* from Puerto Rico, Nigeria, Cuba and Papua New Guinea; *M. javanica* from Fiji, Tonga, Venezuela, Colombia, Tanzania and Florida, USA. *Meloidogyne* spp. are also reported on tannia from Kiribati and Western Samoa in the Pacific and from Trinidad and Jamaica (Bridge *et al.*, 2005). *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* were all found occurring on *Xanthosoma* in Uganda, with *M. hapla* in greater mean densities (Coyne *et al.*, 2003).

M. incognita has been found in high densities causing galling and roughening of the surface of *Xanthosoma* corms (Acosta, 1979). Similarly, *M. javanica* can cause obvious corm damage (Orton Williams, 1980). *M. arenaria* can cause

severe galling and malformation of *X. sagittifolium* corms (Decker and Casamayor, 1966). *Meloidogyne* has also been reported in association with stunting and yellowing of *Xanthosoma* plants, with nematode galls localized at root tips (Roman, 1978). However, most findings indicate that *Xanthosoma* spp. are generally tolerant of *Meloidogyne*, except when pre-plant densities are high (McSorley *et al.*, 1983a). Initial soil densities of 5000 *M. incognita* juveniles/l soil can reduce corm weight of *X. sagittifolium*, but nematode densities decline to 14/l of soil at harvest, indicating that it is a poor host (Caveness *et al.*, 1981). In Tanzania, however, *X. sagittifolium* was found to be a good host of both *M. javanica* and *M. arenaria*, where they occurred at densities up to 6570 J2/g dry root tissue (Runkulatile and Teri, 1990). Infected roots showed black to brown lesions and stubby root characteristics, but no gall formation.

It has been suggested that *M. incognita* is involved in a *Xanthosoma* root rot disease in Papua New Guinea (Bridge and Page, 1984).

Rotylenchulus reniformis

The reniform nematode *R. reniformis* is reported on *Xanthosoma* spp., sometimes in high densities, in the Pacific islands of Fiji, Kiribati, Western Samoa, Tonga and Papua New Guinea, and also from Puerto Rico, Trinidad, Florida and Uganda (Bridge *et al.*, 2005).

Soil densities of 400 *R. reniformis*/100 cm³ soil can cause reduction in root weight and a 26% reduction in the dry weight of marketable cormels of *X. caracu*. The same levels of infection did not affect the yield of *X. atrovirens* (McSorley *et al.*, 1983a). Densities of 100–1000 nematodes/100 cm³ soil have been found associated with small root lesions on *X. sagittifolium* (Brathwaite, 1972). In Fiji, *R. reniformis* occurred in 80% of *X. sagittifolium* plantings (Orton Williams, 1980), but tannia was a non-host for the nematode in a host range trial (Vilsoni and Heinlein, 1982).

The amount and type of damage caused by *R. reniformis* will depend on the species and cultivar of *Xanthosoma*, as well as on soil nematode densities. Nematode control has been recommended only in sites heavily infested by *R. reniformis*, but not where densities are low (McSorley *et al.*, 1983a).

Pratylenchus

Pratylenchus spp. have been recorded on *X. violaceum* in Honduras (Pinochet and Ventura, 1980) and on *X. sagittifolium* in Fiji, Tonga and Western Samoa (Orton Williams, 1980). In Fiji, *P. coffeae* was associated with 50% of *Xanthosoma* plants examined, occasionally present in the outer corm layers in areas around the margin of blackened rotted tissue.

Helicotylenchus multicinctus

Helicotylenchus multicinctus was associated with cocoyam in Ghana (Addoh, 1971), and was found regularly in the roots of *Xanthosoma* plants in Uganda (Coyne *et al.*, 2003). However, no damage has been associated with *H. multicinctus*.

Other Root and Tuber Crops

There are over 27 species of minor root and tuber crops that are of local importance in several tropical and subtropical regions of the world (Kay, 1987). Nematological information is not available for most of these crops. Those crops on which some nematological investigations have been assessed are giant taro (*Alocasia* spp.), giant swamp taro (*Cyrtosperma chamissonis*), Chinese water chestnut (*Eleocharis dulcis*) and crops in certain tropical regions of Central and South America: oca, *Oxalis tuberosa*; olluco, *Ullucus tuberosus*; arracacha, *Arracacia xanthorrhiza*; and mashua, *Tropaeolum tuberosum*, which constitute the basic diet of the population.

Giant taro

Giant taros (*Alocasia* spp.) are grown for their large edible corms. The most common species is *Alocasia macrorrhiza*. A number of plant parasitic nematodes have been isolated from around *Alocasia* plants, but there is no information on their importance. Most records come from the Pacific (Orton Williams, 1980). Two species of *Meloidogyne*, *M. javanica* and *M. arenaria*, have been

reported causing root galls on *Alocasia* sp. in southern Africa (Martin, 1969).

Giant swamp taro

The giant swamp taro, *C. chamissonis*, is a crop of the Pacific Islands grown in flooded swamp for its large edible corms.

There are few records of plant parasitic nematodes associated with *Cyrtosperma*. *Criconeoides denoudenii*, *Criconeoides onoensis*, *H. dihystrera*, *Meloidogyne* sp. and *P. coffeae* have been found around plants in Fiji (Orton Williams, 1980). The burrowing nematode *R. similis* appears to be the main nematode problem, causing a rot of corms in the Pacific islands of Yap, Palau and Guam (Jackson, 1987; Murukesan *et al.*, 2005). Nematode-infected plants seldom show any above-ground symptoms, but corms have small shallow holes, no more than 1–2 cm deep for the most part, except in severe instances, when the entire basal part of the corm is decayed. The rot is wet, with a loose mass of brown dead tissues and a deep brown necrotic centre housing nematodes inside. Usually, the infected tissues spread a disgusting odour typical of this disease. The dead tissues progress into shallow to deep cavities that advance towards the edible central portion of the corm, giving a perforated appearance on the outside of the otherwise smooth corm (Fig. 8.14). The market quality of the corm is reduced greatly by the nematode damage.



Fig. 8.14. *Radopholus similis* damage to swamp taro (*Cyrtosperma chamissonis*) corm surface on the left and subsurface, with slice removed on the right, in Yap, South Pacific. (Photograph courtesy of J. Bridge, from material collected by G.V.H. Jackson.)

Chinese water chestnut

Chinese water chestnut (*E. dulcis*) is commercially cultivated in South-east Asia, the Pacific and southern USA for its edible corms. *Dolichodorus heterocephalus*, the awl nematode, is reported to reduce growth of the crop in the USA (Tarjan, 1952).

Oca and olluco

Oca, *O. tuberosa*, is an important crop of the cold areas of the Andes, grown as a minor crop at elevations over 3000 m from Bolivia to Venezuela, considered of less importance than potato, but more important than olluco. There are several kinds of oca: the bitter, which has white tubers, and the sweet, with tubers of various colours. Because of the high content of calcium oxalate in the tubers, they can only be eaten after days of exposure to sun.

Olluco, *U. tuberosus*, is endemic to the Andes and constitutes a staple food crop for the region between Bolivia to Colombia, after potato and oca.

Several nematode species are associated with oca and olluco (Jatala, 1989). *Atalodera* (= *Thecavermiculatus*) *andina* and *Nacobbus aberrans* are widely distributed in the areas of oca and olluco cultivation (Aztocaza Perez, 1981; Jatala, 1989; Lax *et al.*, 2006). Although roots of these crops are severely infected by *A. andina* and *N. aberrans*, their economic importance as production constraints is not well known. The reactions of these crops to *A. andina* and *N. aberrans* indicate the possibility of an available resistant gene base. *Meloidogyne* species are often found in association with *A. andina* and *N. aberrans* on the roots of these crops, but do not appear to constitute a major constraint.

Arracacha

Arracacha, *A. xanthorrhiza*, belonging to the family Apiaceae (Umbelliferae) is a perennial herb, native to areas from Mexico to Peru. Its fleshy tubers constitute an important food item among the people of Central America and the Andean regions. It has various names throughout Latin America: Árracacham zanhoria blanca in Peru, Manidquina Salsa in Brazil and

Peruvian carrot. From the Andean region of South America, its centre of origin, it was introduced successfully to mountainous regions of Brazil and Central America and, recently, to India and eastern Africa. Colombia is probably the largest producer of this crop. Due to its rusticity and excellent quality starch, it is expanding rapidly into a commercial crop in several provinces in Brazil, with average yields of 10 t/ha.

Nematodes

Of the nematode species attacking arracacha in South America, *Meloidogyne* spp. are of major importance (Jimenez *et al.*, 2001; Henz, 2002). Severe and early root infection inhibits the development of tubers. Infected plants exhibit general symptoms of stunting, yellowing and a tendency to wilt readily during hot, dry periods (P. Jatala, unpublished). In Brazil, both roots and tubers of arracacha can be severely infected by *M. hapla* and *M. incognita*, rendering tubers unmarketable, resulting in a 100% loss.

Arracacha lends itself to high *Meloidogyne* multiplication rates due to its long (10 months) vegetative cycle, which favours multiple generations of the nematode. Thus, farmers need to take care in selecting which crop follows arracacha; two consecutive crops of arracacha are to be avoided. Prior to planting, poor host crops to the nematode should be planted, such as mucuna or *Stylobium* spp.

Corm disinfection can be effective by immersion in a 1% sodium hypochlorite solution for 15 min, then drying under ambient temperatures. Few sources of resistance have been detected, although cultivars with white-coloured roots are considered to be more resistant than those with yellow roots.

The lesion nematode, *Pratylenchus penetrans*, also causes small to large lesions of these organs, which can penetrate deep into the tuberous roots (de Mendes *et al.*, 2001). Suggested control is by nursery soil treatment and the use of nematode-free planting material (Lordello, 1981).

Mashua

Mashua, or aflu, *T. tuberosum*, probably originated in the altiplano zones of Peru and Bolivia. It is an annual crop that produces cone-shaped

tubers similar to oca in form and colour. It is the least popular of the tubers and root crops of the region. The tubers are not palatable when eaten raw and must be sun-cured prior to cooking. They are also dehydrated to form 'chuno', as with potatoes, oca and ulluco.

Of the nematode species attacking this crop, *N. aberrans* and *Meloidogyne* spp. are of major importance, and *N. aberrans* can become a limiting factor to production (Jatala, 1989). However, its economic damage to mashua production is unknown.

Future Prospects

Root and tuber crops constitute a major food source for a great part of the world's population. Given that these crops are largely propagated vegetatively and that a major carry-over of

nematode pests, between cropping seasons, occurs through such planting material, there is need to ensure a focused attention to address these nematode problems. The development of sustainable healthy seed production systems is essential to overcoming nematode and other pest and disease problems of these crops, increasing availability and access to good quality, healthy material – towards their improved production. The assessment of nematode problems on minor root and tuber crops and their economic importance in production systems requires greater attention. Although root and tuber crops constitute the basic staple diet of much of the world's population, nematological information regarding these crops is sorely lacking. With the intensification of root and tuber crops and the monocropping of high-yielding cultivars, such as cassava for industrial purposes, nematode problems are likely to become increasingly important production constraints.

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9 Nematode Parasites of Food Legumes*

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The family Leguminosae is the third largest family of flowering plants in the world, with 751 genera and more than 19,000 species. However, less than 20 species are of worldwide economic importance as food crops, whereas another 200 are considered important on a regional or local basis (NAS, 1979). For practical purposes, legume crops are often grouped under a variety of names, including: legumes, pulses, grain legumes or beans. The use of any one term is misleading, because these crops can often be used simultaneously as a grain, vegetable, green manure, pasture or cover crop, source of fodder, as well as for cooking oil or as a protein supplement. Therefore, we have decided to use the broader term 'food legumes' for the crops discussed in this chapter. Economically, legumes represent the second most important crop family after Poaceae (grass family), accounting for approximately 27% of the world's crop production (Graham and Vance, 2003).

Over the past 15 years, there has been a major increase in the level of production of almost all legumes (Table 9.1), with massive increases in the level of production of soybean, dry bean, groundnut, chickpea and cowpea. The many uses of legumes not only for direct human consumption but also as animal feed and as raw material for food processing has been responsible for this enormous expansion in production

worldwide, as can be seen in the increase in production of soybean. Of course, the steady increase in world population from 6 to 11.2 billion between 2000 and 2016 has also influenced the expansion of food legumes. The main climatic zones, uses, distribution and relative economic importance of the most important food legumes are presented in Table 9.2.

Legumes rank second to cereal crops in degree of nutritional importance for humankind. In many countries, they are the major source of protein, often containing two to three times more protein than cereals. On a global basis, one-third of the protein consumed by humans comes from food legumes, and an estimated 80% of the protein in the diet of many tropical and subtropical countries is derived from vegetable products, among which food legumes predominate. Over 50% of the food legumes are produced and consumed in Asia. In India, they supply the only high-protein component of the diet (Kay, 1979). Legumes are the cheapest and most direct form of protein. They can be transported easily when dried and can be stored for long periods at room temperature without losing substantially on nutritional content.

The negative impact of plant parasitic nematodes, insects, plant diseases and abiotic disorders are reflected in the relatively low yields in tropical agriculture when compared with yields

*A revision of the chapter by R.A. Sikora, N. Greco and J.F.V. Silva in the second edition.

Table 9.1. Common name, growing zone, use, distribution and importance of food legumes in tropical and subtropical climatic areas. (From Brothwell and Brothwell, 1969; NAS, 1979; Ward *et al.*, 1981.)

Common names	Climatic zones ^a				Uses			Distribution	Importance
	T	ST	UT	D/SA	Grain	Veg.	Animal ^b		
Adzuki bean		x			x		x	Worldwide, China, Japan, SE Asia	++
Black gram, urd	x	x	x		x			India	++
Broad bean, faba bean	x	x			x	x	x	Worldwide	+++
Catjang bean	x	x				x	x	Worldwide, SE Asia	++
Chickpea	x	x		x	x	x	x	Worldwide	+++
Cowpea	x	x		x	x	x	x	Worldwide	+++
Grass pea, chickling pea		x	x		x		x	Worldwide	+
Haricot, kidney, bush, French, string bean	x	x			x	x		Worldwide	+++
Horse bean	x			x	x		x	Asia, Africa	+
Horse gram	x			x	x		x	Asia, Africa	+
Hyacinth bean, lablab	x	x		x	x		x	SE Asia	++
Lentil		x	x	x	x		x	Worldwide	+++
Lima bean, butter bean	x	x			x	x		Worldwide	++
Lupin, tarwi		x			x		x	N. and S. America, Mediterranean	+
Moth bean	x	x		x	x		x	Worldwide, India, S. America	+
Mung bean, green gram	x	x			x	x	x	Worldwide, India, China, SE Asia	+++
Pea		x	x		x	x	x	Worldwide	+++
Pigeonpea, red gram	x	x		x	x	x	x	Worldwide	++
Rice bean, red bean	x	x			x		x	Worldwide, SE Asia	++
Soybean, soya	x	x			x	x		Worldwide	+++
Winged bean	x				x	x	x	Worldwide, SE Asia	+

Notes: ^aT = tropical; ST = subtropical; UT = upland tropics; D/SA = dry/semi-arid tropics. ^bAnimal = fodder, green manure, protein supplement or straw.

Table 9.2. World food legume production in t × 1000 in 2000 and 2014. (From FAOSTAT, 2016; <http://www.fao.org/faostat/en/#data>.)

Legume crop	2000	2014
Soybean	161,299	308,436
Dry bean	17,654	25,094
Green bean	9,874	21,366 ^a
Broad bean	3,722	4,297
Chickpea	8,009	14,239
Cowpea	3,267	5,589
Groundnut	34,742	42,444
Lentil	3,369	4,885
Pigeonpea	3,259	4,858
Pulses nes ^b	3,296	5,152

Notes: ^aData from 2013. ^bPulses nes = lablab or hyacinth bean (*Dolichos* spp.); jack or sword bean (*Canavalia* spp.); winged bean (*Psophocarpus tetragonolobus*); guar bean (*Cyamopsis tetragonoloba*); velvet bean (*Stizolobium* spp.) and yam bean (*Pachyrhizus erosus*).

in temperate zones. There is also a major difference in yield between regions where modern agronomic practices are prevalent versus those where subsistence agriculture predominates. The difficulty associated with pest and disease management in legumes is also seen in the lack of significant yield improvement between 2000 and 2016 when compared to other grain crops (FAOSTAT, 2016). The symbiotic relationship between legumes and nitrogen-fixing *Rhizobium* bacteria gives these crops an economic advantage over crops requiring fertilizer. Part of the fixed nitrogen remains in the soil within crop residues after harvest, thus improving soil fertility when reincorporated. Food legumes are, therefore, an important component in tropical cropping systems, where they are rotated with such nutrient-demanding crops as rice and maize. In many tropical and subtropical regions,

soils are highly degraded and deficient in organic matter. In these areas, legumes are being used not only for food production but also to help improve degraded soil, especially when used as green manure.

Nematodes of Food Legumes

Many plant parasitic nematodes have been found associated with legume crops and are discussed in this chapter. However, only those nematodes that are known to cause yield loss are featured here and in previous revisions of this chapter. Nematodes that are only known to parasitize and complete their life cycle on the plant, but do not cause significant damage, will not be discussed in detail. These associations are, however, noted in Table 9.3.

When food legumes are cultivated under conditions of drought and/or heat stress in semi-arid areas, nematode-infected plants are often severely damaged. In these areas of the world, where legumes are also often grown on degraded soils with marginal inputs, nematodes can add significantly to yield depression. Nematodes induce vascular disorders and reduce root penetration of the soil profile by infecting and inhibiting primary root development of the seedling. The resulting nematode-induced shallow root architecture increases the negative impact of moisture stress on root health and yield (Sikora, 2016). Sikora suggested that breeding programmes needed to be improved to offset the impact of nematodes on root growth under moisture and heat stress conditions.

Plant parasitic nematodes also affect plant vigour in some food legumes by suppressing *Rhizobium* root nodulation and nitrogen-fixing activity. Complex interrelationships between nematodes and soil-borne fungal pathogens, therefore, also play a significant role in reducing yield. The importance of these complex interrelationships has received only minor attention.

Broad Bean

Broad bean (*Vicia faba*), also known as faba, field, common, horse, tick and Windsor bean, is a subtropical or temperate crop that is probably

native to the Mediterranean region or South-west Asia. It is grown in the winter season in the subtropics. The dried seeds are eaten as porridge or consumed after baking as fowl in the Middle East, whereas fresh, immature seeds are consumed as a vegetable after boiling. The seeds are also widely used as livestock and poultry feed. The crop is sometimes used as green manure and the dried residues as animal fodder.

A wide range of plant parasitic nematodes have been found associated with *V. faba*, but only a few are of widespread economic importance in the tropical and subtropical zones (Hooper, 1983b). In most cases, nematode damage occurs in the cooler winter growing seasons in the subtropics or in the upland tropical zones.

Ditylenchus

The stem nematode, *Ditylenchus dipsaci*, is the most important nematode on broad bean in subtropical and temperate growing areas. The nematode has been detected attacking broad bean in many countries bordering the Mediterranean Sea, including Syria, Jordan, Turkey, France, Tunisia, Algeria, Morocco, Cyprus, Spain, Italy and Greece, and more recently in Iran (Fasihi *et al.*, 2010; Maafi, *et al.*, 2013). Because of the nematode's worldwide distribution, it should be considered a potential pest in most areas where broad bean is grown (Hooper, 1972; Lamberti, 1981; Greco and Di Vito, 1987; Sellami, 1998; Abbad and Bachikh, 2001; Troccoli and Di Vito, 2002).

Biology

The stem and bulb nematode is a migratory endoparasite that feeds on stem, petiole, leaf, pod and seed tissue (see Chapter 2, this volume). The nematode does not cause damage to the root. Soil-borne *D. dipsaci* fourth-stage juveniles penetrate the young seedling below the soil surface after germination. Damage is often more severe when seed-borne populations are already present in the tissue at planting. Cool, moist conditions during the winter growing season in the Mediterranean region favour nematode infection and disease development. As temperatures rise during the growing season, nematode development is slowed, resulting in symptoms disappearing due to lack of nematode feeding activity.

Table 9.3. Plant parasitic nematodes associated with food legumes of local or limited importance in tropical and subtropical climatic areas. (From Sikora *et al.*, 2005.)

[illegible]

Survival and means of dissemination

The fourth-stage juvenile can withstand desiccation for many years. The nematodes often clump together to form 'nematode wool' when the plant tissue begins to dry. This wool can often be observed on the seeds in heavily infested pods. The presence of desiccated infective fourth-stage juveniles in seed as well as in plant debris is important in the passive dissemination of the nematode over long distances. *D. dipsaci* is seed borne in broad bean, lucerne, onion, clovers and teasel. The nematode in this desiccated stage can survive passage through pigs and cattle on infested seed (Palmisano *et al.*, 1971; Augustin, 1985).

Although nematode soil densities seem to decrease rapidly, Seinhorst (1956a) and Wilson and French (1975) show that the nematode can survive for years without a host plant. However, many weeds and grasses are host for the nematode and may play an important role in its survival in the absence of cultivated plants. Nematode survival and damage are greater in heavy soils as compared with sandy soils (Seinhorst, 1956b). Hooper (1972) estimated that the nematode would die out within 8 years in the absence of a host, a timespan that rarely occurs in present-day agriculture.

Races

Broad bean is attacked by the normal 'oat race' (1.2–1.4 mm adult or fourth-stage juvenile body length) in temperate regions and by the 'giant race' (1.5–1.7 mm length) in the subtropical semi-arid regions of the Mediterranean. These two races can be distinguished on the basis of chromosome number; $2n = 24$ in the 'oat race' versus double that number in the 'giant race'. Recently, these two races were also distinguished by molecular methods (Esquibet *et al.*, 1998). There are other races that can attack broad bean, but they are of marginal importance in temperate regions.

Kerkoud *et al.* (2007) have identified specific primers for the identification of races in the *D. dipsaci* species complex that have potential use in quarantine. More recently, Vovlas *et al.* (2011) described the 'giant race' as a new species (*Ditylenchus gigas* n. sp.) that is clearly distinguishable from the smaller 'oat race' (*D. dipsaci sensu stricto*) based on morphological and molecular data obtained from several Mediterranean populations.

The fact that *D. gigas*, or the former 'giant race', causes damage in England (Hooper, 1983a) and can survive under environmental conditions existing in most temperate regions of Europe warrants closer examination of imported broad bean seeds originating from subtropical growing areas in all regions of the world.

Symptoms

Although Hooper (1983a) suggested that the two races could be identified tentatively by the symptoms produced, with the more severe symptoms being induced by *D. gigas*, known formerly as the 'giant race', he considered measurement of body length a more exact means of identification. The nematode can induce stem swelling and deformation of stem tissue (Fig. 9.1), or lesions that turn reddish-brown then black, depending on the cultivar and environmental factors. The lesions envelop the stem and increase in length, often advancing to the edge of an internode. Leaf and petiole necrosis is also common under heavy



Fig. 9.1. Darkened, reddened stems on broad bean, *Vicia faba*, infested with *Ditylenchus gigas* in Syria, showing reduced tillering. (Photograph courtesy of R.A. Sikora.)

infestations, but can be confused with symptoms produced by fungal leaf pathogens. Newly formed pods take on an even, dark brown appearance (Hooper, 1983a). Seeds infested with the nematode are darker, distorted, smaller in size and may have speckle-like spots (Fig. 9.2.) on the surface (Schreiber, 1977; Hooper, 1983b; Augustin, 1985). The percentage of seeds infested increases with infestation levels, and is greatest when nematode-contaminated seed is used for sowing. Heavy infestations often kill the main shoot, which stimulates secondary tiller formation (Fig. 9.3). These newly formed shoots are often free from infection. The nematodes are found under the testa



Fig. 9.2. Speckled, blackened lesions on *Vicia faba* seeds infested with *Ditylenchus gigas*. (Photograph courtesy of R. Sikora.)



Fig. 9.3. Increased tiller production in *Ditylenchus gigas*-infested *Vicia faba* in Syria. Notice darkened stems at the base of the plant. (Photograph courtesy of R. Sikora.)

in depressions on either side of the radicle, causing necrotic patches, visible when the testa is removed (Hooper, 1983b). Over 10,000 juveniles can be found in one infested seed.

It should be noted that Caubel and Leclercq (1989) observed that two types of symptoms developed following infection; that is, swelling and shortening of the inoculated axillary bud in resistant plants, and necrotic lesions surrounding the inoculation site in susceptible lines.

Economic threshold level

Hooper (1983a) in field trials showed that the 'giant race' (now *D. gigas*) was more damaging to broad bean than the 'oat race', common to Europe, when *D. dipsaci*-infested straw was incorporated into the field. The 'giant race' caused 100% and 63% and the 'oat race' 82% and 1.3% stem and seed infection, respectively. The economic threshold level is not known for *D. gigas* on broad bean. The threshold levels for the 'oat race' on onion, celery and carrot is 2 nematodes/100 g of soil (Decker, 1969).

Other hosts

Although *D. dipsaci* has over 450 host plants (Hooper, 1972), the host range of the 'giant race' seems to be more limited. *D. gigas* is usually very damaging to wild oats, and some Moroccan populations are also injurious to pea (M. Di Vito, Italy, 2003, personal communication). Eleven out of sixty weed species found in fields of broad bean in Morocco were infested with *D. gigas*. In addition to weeds, *Avena sterilis*, *Vaccaria pyramidata* and *Verbena supia* were good hosts, as well as the economically important parasitic weed of broad bean, *Orobancha crenata* (Abbad and Bachikh, 2001). The 'oat race' has also been reported to attack chickpea, pea and lentil in the Mediterranean basin, whereas the 'giant race' multiplied on faba bean but only infested the stems of lentil and vetch (Caubel *et al.*, 1998b). Certain weeds serve as hosts for the 'oat race' (Green, 1981) and *D. gigas*, the 'giant race' of *Ditylenchus* (Augustin and Sikora, 1989a), and are important in maintaining high soil densities of the nematode. In South Australia, the 'oat race' also affected the emergence of canola (*Brassica napus*). Inoculated seedlings showed typical symptoms of damage, whereas both

tolerance and resistance were observed in mature plants (Taylor and Szot, 2000).

Management measures

Prevention of introduction by establishing quarantine laws should be promoted. Seeds can be examined easily by the techniques outlined below and in Chapter 4, this volume. Rotation of at least 4 years with non-host crops and control of weed hosts is required for successful management. Rotations of 3 years will reduce damage significantly compared with 2 years between crops. Caubel *et al.* (1998a) demonstrated that infection by the 'oat race' was always associated with previous cropping of broad bean, pea or fodder beet, but not maize.

Fumigation has been used to eradicate the nematode from infested seeds, but will not give 100% control when high infestation exists (Powell, 1974; Augustin, 1985). Soil treatment with non-fumigant nematicides will prevent seed infestations and can be used to protect breeding material (Augustin and Sikora, 1984; Augustin, 1985).

D. gigas was known from Egypt (B.A. Oteifa, Egypt, 1997, personal communication), but was not detected in a survey in Egypt by Augustin (1985). The nematode was also reported on a local Moroccan cultivar by Schreiber (1977) and in Syria by Hanounik *et al.* (1986).

Good levels of resistance to the *D. gigas* 'giant race' have been detected in breeding lines (Sikora *et al.*, 2005), but, to our knowledge, commercial cultivars are not yet available for use. It should also be noted that the production of uninfested tillers after the main stem is killed by the nematode may be confused with resistance (Hooper, 1983a).

Heterodera

The pea cyst nematode *Heterodera goettingiana* is an important parasite of broad bean in many temperate regions. The nematode is a limiting factor in the cool growing season in some countries of subtropical North Africa, West Asia, Italy and Spain (Stone and Course, 1974). The nematode causes stunting in heavily infested fields (Fig. 9.4).



Fig. 9.4. Broad bean crop showing a patch of stunted plants in a field infested with *Heterodera goettingiana*. (Photograph courtesy of N. Greco.)

Other hosts

Most host plants are in the tribe Viciae of the family Leguminosae. *Pisum sativum*, *Lathyrus* species and species of *Vicia* as well as soybean are considered economically important hosts for *H. goettingiana* (Jones, 1950; Winslow, 1954). However, because soybeans are a summer crop, the high temperatures during the growing season result in *H. goettingiana* rarely being of economic importance on this host. Lentil (*Lens culinaris*) is reported to be a host, but the authors believe this is by mistaken identity in that Tedford and Inglis (1999) found this crop to be a very poor to non-host to the pea cyst nematode.

Lentil was reported a host for *H. goettingiana* in the Irbid area of Jordan, but there is no evidence that the nematode was identified properly. In the same area in the early 1990s, lentil was infested by *Heterodera ciceri*, which was probably the nematode observed earlier on lentil (N. Greco, Italy, 2003, personal communication). In addition, many weeds are considered good hosts of the nematode and are responsible for maintaining populations in the absence of susceptible crop plants.

Biology

The biology and development of this nematode are similar to those described for the other cyst nematodes at the beginning of this chapter and in Chapter 2, this volume. *H. goettingiana* only completes one generation per growing season if only cysts and not egg masses are produced (Fig. 9.5), but multiple generations can be produced on both broad bean (Hooper, 1983a) and garden pea (Greco *et al.*, 1986a) sown in early autumn, when egg masses are formed. Survival in the absence of a host has been reported to exceed 10 years (Brown, 1958).

Economic threshold level

The threshold level of nematodes on broad bean is 0.8 eggs/g of soil, with complete crop failure occurring at 64 eggs/g of soil (Greco *et al.*, 1991). The crop is, however, less susceptible to damage than pea. Growing the crop every 4 years in infested fields caused crop failure under temperate climatic conditions (Brown, 1958), but resulted in a good crop stand under Mediterranean conditions (Di Vito and Greco, 1986).



Fig. 9.5. Broad bean roots, *Vicia faba*, with massive numbers of white *Heterodera goettingiana* cysts from an infested field in Jordan. (Photograph courtesy of J. Bridge.)

Management measures

Effective control can be obtained by crop rotation with non-host crops. On uninfested land, Hooper (1983a) recommended reducing legume crops to once in 4 years. Where severe infestations are known, longer rotations are required (Brown, 1958). *Cicer arietinum*, *Glycine max*, *Lupinus albus*, *Medicago sativa*, *Phaseolus vulgaris* and a number of clover species were found to be resistant to the nematode (Di Vito *et al.*, 1980; Tedford and Inglis, 1999). Nematicides have been shown to be effective in controlling *H. goettingiana* on peas, but have not been examined for use on broad bean. Seed treatment with nematicide could be an alternative management option.

Other nematodes of broad bean

There are a number of other nematodes that parasitize broad bean in the tropics and subtropics that are of local, limited or unknown importance.

The root knot nematodes *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne arenaria* and *Meloidogyne artiellia* are known to attack

broad bean in the tropics and subtropics. Damage caused by root knot nematodes has been observed in Italy (N. Greco and M. Di Vito, Italy, 1989, personal communication) and Sicily (Lombardo *et al.*, 2011), as well as in Zimbabwe, Malawi, East Africa, Libya and Iraq (Hooper, 1983a). The symptoms of damage and methods of diagnosis are the same as those described for other legumes in this chapter. There is also an indication that the nematode can reduce nodulation (El Bahrawy and Salem, 1989). Control is usually accomplished by rotation with non-host crops, especially cereals. Care should be taken in selecting rotation crops, because of the nematodes' wide host range and known variability in the genus. Resistance has not been identified and nematocides are too expensive for practical use.

Some species of *Pratylenchus* cause extensive necrosis of the root tissue and yield loss in the subtropics and tropics. The impact of this group of nematodes to broad bean, however, has not been determined. Broad bean is a good host for *Pratylenchus neglectus*, *Pratylenchus penetrans*, *Pratylenchus pinguicaudatus* and *Pratylenchus thornei* (Di Vito *et al.*, 2002a), and some broad bean lines were found to be resistant to these species (Di Vito *et al.*, 2002b). In pot experiments, the tolerance limit of this legume to *P. neglectus* and *P. thornei* was 2 nematodes/cm³ of soil (Di Vito *et al.*, 2000).

The burrowing nematode *Radopholus similis* has been shown to reproduce on broad bean only in India (Sosamma and Koshy, 1977). *Rotylenchulus reniformis* has been reported on broad bean in Pakistan (Timm, 1956) and from Egypt, where screening of germplasm demonstrated the existence of resistance (Ismail and Amin, 2013). Hooper (1983a) discussed the distribution and importance of stunt nematodes in the family Tylenchorhynchidae on the crop. However, stunt nematodes are considered of limited economic importance on this crop worldwide.

Chickpea

Chickpea (*C. arietinum*), also known as gram, Bengal gram, garbanzo, Egyptian pea or Kabuli chana, originated from Turkey and Syria around 5450 BC (Saxena, 1987). Production is concentrated in Asia, where 84% of the world's crop is grown, with India accounting for about 57%

of the area in cultivation. Other countries with extensive cultivation are: Pakistan, Myanmar (Burma), Iran, Ethiopia, Mexico, Canada and Australia. In the Mediterranean basin, chickpea is an important crop in Turkey, Syria, Morocco, Tunisia, Spain and Portugal. Although the green pods and shoots of chickpea are also used for vegetables in India, this legume is used mainly as dried grains, which are boiled, mashed or roasted, and used for flour in various foods. A minor portion is used as animal feed.

Two types of chickpea are commonly grown: (i) Desi – small seeded with a brown seed coat common to India and used for flour, 'dhal' (an important split-pea vegetable) and to a lesser extent as animal feed; and (ii) Kabuli – large seeded with a thin, light-coloured seed coat and usually consumed whole in West Asia.

Chickpea is moderately resistant to drought and sensitive to low temperature; therefore, it is cultivated as a winter crop in India, Pakistan and Australian coastal areas, and as a spring crop in Turkey, Syria, Ethiopia and Canada. Winter chickpea yields are nearly double the amount of spring chickpea. Chickpea can be cultivated successfully in areas with a minimum annual rainfall of 300 mm. Supplementary irrigation may double yields. Chickpea is irrigated in the Nile Valley of Egypt and Sudan, due to a lack of sufficient rainfall, and in India in areas where soils have low water-holding capacity (Saxena, 1987).

Chickpea infected by nematodes are, in general, stunted, with chlorotic foliage. They flower poorly and give rise to a few small pods that are often empty. Crop senescence sets in earlier than normal in heavily infested fields. The root system of infected plants is reduced in size; *Rhizobium* nodulation is suppressed and roots can show extensive necrosis. Since these symptoms are not specific to the nematodes, close examination of the root system is required for proper diagnosis. The nematodes associated with chickpea have been reviewed by Sharma (1985) and Ali (1995).

Meloidogyne

M. javanica, *M. incognita* and *M. arenaria* damage chickpea in India (Mathur *et al.*, 1969; Nath *et al.*, 1979) and *M. arenaria* in Ghana (Edwards, 1956). *M. javanica* was also found on chickpea in the Ethiopian highlands. Infected plants have

heavily galled roots that may rot due to invasion by fungal pathogens. The concomitant presence of *M. incognita* and *M. javanica* may enhance the severity of the soil-borne fungus *Fusarium oxysporum* f. sp. *ciceri* (Siddiqui and Mahmood, 1994; Maheswari *et al.*, 1997; Charu Jain and Trivedi, 1998). Moreover, chickpea lines may lose their resistance to the fungus when infested with these root knot nematodes (Maheswari *et al.*, 1995; Rao and Krishnappa, 1996). The nematodes may also infest and develop on *Rhizobium* nodules that senesce earlier (Vovlas *et al.*, 1998).

In the subtropical semi-arid Mediterranean basin, damage is conspicuous when chickpeas are planted in sandy-loam soils in late summer or early autumn. Conversely, crop injury is minimized when chickpeas are sown from late autumn into the winter season. Soil temperatures suitable for nematode attack and development are not reached until late spring, allowing the plant to escape the damaging early root invasion process. For this reason, root knot nematodes, although important on other summer crops, do not constitute a problem in the Mediterranean basin.

The nematodes, however, are a serious problem in tropical zones. Their importance was demonstrated in India by Upadhyay and Dwivedi (1987), who treated field plots infested with 4.6 *M. incognita* juveniles/cm³ with a nematicide and observed increases in yield of 40%. Yield losses of 31–37% were detected in nematode trials when *M. incognita* was present at 2.5 juveniles/g of soil (Reddy, 1985), and Ali (1995) reported yield reduction up to 60%.

Economic threshold level

In pot experiments, the growth of chickpea was affected negatively when soil populations of *M. incognita* (Nath *et al.*, 1979) and *M. javanica* (Srivastava *et al.*, 1974) exceeded 0.2 juveniles/g of soil. Ahmad and Husain (1988) detected reductions in shoot length and total plant weight at densities of 1 juvenile/g of soil in pot studies. However, under field conditions, yield losses differ greatly between countries. This variation is caused by differences in soil type, environmental factors existing during the growing season in the different climatic zones and complex disease interrelationships. Therefore, field studies are

required to estimate tolerance limits and make yield loss assessments.

Management measures

Crop rotation, including fallow, is currently used to control root knot on chickpea. Rotation is complicated by the wide host range of species of *Meloidogyne*. Nevertheless, groundnut (peanut) and winter cereals are non-hosts for *M. incognita* and *M. javanica*, and cotton is a non-host for *M. incognita* and *M. arenaria*. Saka and Carter (1987) have listed hosts and non-hosts of *M. incognita*.

Sowing in late autumn, when soil temperature drops below 18°C, and harvesting in spring can limit or prevent nematode reproduction (Roberts *et al.*, 1981). Chickpea should not be planted in early autumn in fields planted the previous summer to a susceptible host crop. In India, postponing sowing to late autumn has been shown to suppress yield loss (Gaur *et al.*, 1979).

Meher *et al.* (2008) demonstrated the positive effects of nematicidal seed treatment on *M. incognita* that was cost effective. Although soil application of nematicides can reduce nematode damage, they cannot be used economically on chickpea.

Resistance

Chickpea lines and a few cultivars have been identified as resistant to root knot nematodes. Singh *et al.* (2012) detected moderate to high levels of resistance to *M. incognita* in three germplasm lines.

Meloidogyne artiellia

M. artiellia causes yellowing and stunting of infected plants and severe losses in yield. The nematode was first reported from cabbage in England (Franklin, 1961) and later on chickpea in Spain, Italy (Greco, 1984), Syria (Greco *et al.*, 1992), Turkey (Di Vito *et al.*, 1994b) and North Africa (Di Vito *et al.*, 1994a). The nematode differs significantly from the previously mentioned species of *Meloidogyne* in both morphometrics and ecology. Galls produced on chickpea by Syrian populations of the nematode are indistinct, and almost totally absent in Italian populations.

The most obvious symptom of nematode attack is the presence of large egg masses on the roots. Because of their size, they can be confused with cyst nematode females when observed with the naked eye (Fig. 9.6).

Other hosts

The nematode has a wide host range. Di Vito *et al.* (1985), and many cruciferous, cereal (except oat and maize) and leguminous crops (except lentil, haricot bean, cowpea, lupin, soybean and sainfoin) are good or very good hosts for the nematode. All species in the Solanaceae, Rosaceae, Linaceae, Compositae, Cucurbitaceae, Chenopodiaceae and Umbelliferae plant families were poor or non-hosts.

Biology

Investigations by Di Vito and Greco (1988a) demonstrated that second-stage juveniles could invade chickpea roots at 10°C, but adult stages were not formed after 66 days at this temperature. Nematode development was also retarded

at 30°C. In Italy and Syria, large egg masses can be observed in early April on the roots of chickpea sown the previous autumn, and in early May on spring-sown chickpea. Juveniles may hatch soon after the completion of embryogenesis.

The presence of a combination of insufficient rainfall and high temperature in spring in the Mediterranean basin often causes poor root growth, which limits juvenile emergence from newly produced eggs. This interplay of biotic and abiotic factors is responsible for limiting the nematode to only one generation per growing season. However, if rainfall occurs late in the season, eggs hatch immediately and second-stage juveniles survive during dry and hot summers in an anhydrobiotic condition (Di Vito and Greco, 1988a). The nematode seems to be adapted to a wide range of environmental conditions and develops well in a large variety of soil types, including those types containing 30–40% clay.

Consistent damage is caused to chickpea in Syria, where this crop is rotated with durum hard wheat and barley, both good hosts for the nematode. A survey conducted in the 1980s (Greco *et al.*, 1992) revealed that 13% of chickpea fields in Aleppo province, northern Syria, were infested.

Hernández Fernández *et al.* (2005) observed that the reproductive fitness of five populations of *M. artiellia* collected from Italy, Spain and Syria was significantly greater in durum wheat than in chickpea.

Navas-Cortés *et al.* (2008) reported that co-infection by *M. artiellia* and *F. oxysporum* f. sp. *ciceris* of genotypes resistant to specific races of the fungus did not influence wilt resistance of the plant, irrespective of the *F. oxysporum* f. sp. *ciceris* race assayed. Microplot experiments have shown that chickpea is highly susceptible to nematode attack when population densities exceed 0.14 and 0.016 eggs/cm³ of soil for winter- and spring-sown crops, respectively (Di Vito and Greco, 1988b).

Management measures

The parasite can be controlled effectively by rotating chickpea with non-host crops. In the Mediterranean area, cotton, sugarbeet, potato, oat, maize, lentil, tomato and melon are poor or non-host crops suitable for inclusion in crop rotation management programmes for *M. artiellia*.



Fig. 9.6. Root of chickpea showing large egg masses and indistinct galls caused by infestation of *Meloidogyne artiellia*, which can be confused with cysts of *Heterodera ciceri*. (Photograph courtesy of N. Greco.)

The length of the rotation should be designed to reduce soil densities below threshold levels, which generally requires a 2- to 4-year period with non-host crops. Although nematicides have been shown to be effective experimentally, they cannot be used economically on the crop. No attempts have been made to screen chickpea cultivars and lines for resistance to this nematode.

Heterodera

A cyst nematode infesting chickpea was found in Syria by Mamluk *et al.* (1983), and was observed as the causal agent of severe chickpea decline in Idleb province and other areas in the north of the country (Greco *et al.*, 1992). The nematode was described as *H. ciceri* by Vovlas *et al.* in 1985. The nematode belongs to the *Heterodera trifolii* group and differs from *H. trifolii* in having abundant males, different host range and distinct morphological characteristics (Vovlas *et al.*, 1985; Sikora and Maas, 1986). The nematode has also been detected in several areas of Turkey (Di Vito *et al.*, 1994b), as well as in the Irbid Governorate of Jordan and the Beka'a Valley in Lebanon.

Other hosts

The host range of *H. ciceri* is confined to members of Leguminosae (Greco *et al.*, 1986b). The nematode reproduces well on chickpea, lentil, pea and grasspea (*Lathyrus sativus*) and poorly on *Vicia* spp., haricot bean, lupin and lucerne. However, a Syrian and a Turkish population also reproduced well on lucerne and *Medicago rigidula* (Di Vito *et al.*, 2001). Broad bean and several clovers are poor or non-hosts of the nematode. In tests with plants in 13 botanical families, the nematode produced only a few females on carnation.

Biology

Although the nematode invades chickpea roots at 8°C, development only occurs at temperatures of 10°C and above (Kaloshian *et al.*, 1986a). Root infection is suppressed at 30°C. Females may protrude a small gelatinous matrix, which is void of eggs (Kaloshian *et al.*, 1986b). In the field, large numbers of lemon-shaped white

females can be seen at the beginning of April or 2 weeks later on the roots of winter- and spring-sown chickpeas in the Mediterranean region, respectively. Cysts usually appear 14–16 days later (Greco *et al.*, 1988a), after an accumulation of 370 day degrees above the basal temperature of 10°C (Kaloshian *et al.*, 1986b).

Economic threshold level

The tolerance limit of chickpea to *H. ciceri* is 1 egg/cm³ of soil. Yield losses of 20–50% can be expected in fields infested with 8–16 eggs of the nematode/cm³ of soil, respectively. Complete crop failure occurs in fields infested with ≥60 eggs/cm³ of soil (Greco *et al.*, 1988a). Under field conditions, severe chickpea decline can be observed from the end of April until crop maturity. At harvest, the protein content of chickpea grain produced in infested fields is significantly reduced, thus lowering the nutritional value of the grain.

Management measures

Since this nematode has a rather narrow host range, it can be controlled effectively by crop rotation (Saxena *et al.*, 1992). An annual decline of 50% of the nematode population using non-host crops has been reported (Saxena *et al.*, 1992). These results demonstrated that short 3- to 4-year rotations were effective in reducing the nematode densities to or below the tolerance limit. In the long-term conservation agriculture cereal–legume rotation trials in Syria, neither tillage practices, crops nor planting dates significantly affected *H. ciceri* population densities (Ahmed *et al.*, 2012).

Resistance

None of the nearly 10,000 chickpea lines screened showed resistance to *H. ciceri* (Di Vito *et al.*, 1996; Thompson *et al.*, 2000). However, resistance to the nematode was found in lines of *Cicer bijugum*, *Cicer pinnatifidum* and *Cicer reticulatum*. Because *C. reticulatum* can be crossed with *C. arietinum*, a research programme to introgress the resistance to *H. ciceri* in kabuli-type cultigens is in progress at the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria (Di Vito *et al.*, 1996; Malhotra *et al.*, 2002).

Another cyst nematode, *Heterodera swarupi* (Sharma *et al.*, 1998), was described from roots of chickpea in Rajasthan, India. The nematode belongs to the *Heterodera schachtii* group, is close to *Heterodera cajani* and also can infect pigeonpea. Females of *H. swarupi* turn yellow and produce an egg mass. Recently, *H. swarupi* has been detected in several other districts of Rajasthan, even in large numbers, but its impact on chickpea yield has not yet been assessed (Ali and Sharma, 2003).

Pratylenchus

Root lesion nematodes are migratory endoparasites that cause large cavities and necrosis in the cortex of chickpea roots. Eggs are deposited in the cavities within the root. Several generations may develop in a growing season, each taking about 1 month. Large numbers of lesion nematodes can be extracted from the roots at the early flowering stage of plant development. Plant growth is reduced further through root damage caused by interrelationships with soil-borne root pathogens and adverse effects on *Rhizobium* nodulation. The reduced root system decreases plant resistance to drought conditions, which makes these nematodes important in the dry areas in both the semi-arid and arid regions of the world. In the absence of a host crop, *Pratylenchus* survive in the soil as eggs, juveniles or adults. In dry areas, the nematode survives in an anhydrotic condition (Glazer and Orion, 1983).

The damage these nematodes cause in the field is generally not as severe as that caused by root knot and cyst nematodes. However, severe symptoms of infestation have been observed in Turkey, Lebanon and in countries in North Africa. Because they are found in most fields on a worldwide basis, they are undoubtedly responsible for significant yield loss. Yield losses of 25 and 75% in winter- and spring-sown chickpea, respectively, were observed in Syria in a field infested with *P. thornei* (Greco *et al.*, 1988b).

The most important lesion nematode is *P. thornei*, which has a cosmopolitan distribution. In the Mediterranean region, the nematode was detected in 72% of chickpea fields in Syria (Greco *et al.*, 1992), 61% in Turkey (Di Vito *et al.*, 1994b), 92% in southern Spain (Castillo *et al.*, 1996) and 28–61% in North Africa (Di Vito

et al., 1994a). Under field conditions in Syria, the tolerance limit of chickpea to the nematodes was 0.03 specimens/cm³ of soil, with yield loss of 58% at two specimens/cm³ of soil (Di Vito *et al.*, 1992). In India, population densities ≥ 0.1 /g of soil were responsible for significant growth reduction, while densities of ≥ 4 /g of soil also reduced germination (Walia and Seshadri, 1985). The reaction of chickpea cultivars may differ, and some can be tolerant (Castillo *et al.*, 1998). The nematode appears to reproduce well on cool-season crops and poorly on warm-season crops (Di Vito *et al.*, 1992, 2002a).

Other species of root lesion nematodes found infesting chickpea in the Mediterranean region are *Pratylenchus mediterraneus*, *P. neglectus*, *P. penetrans* and, seldomly, *Pratylenchus crenatus*, *Pratylenchus pratensis*, *P. pinguicaudatus* and *Pratylenchus zeae* (Greco *et al.*, 1992; Di Vito *et al.*, 1994a,b). However, the impact of these species on chickpea has not been assessed.

In Australia, both *P. thornei* and *P. neglectus* are widespread in wheat fields, and they damage chickpea when this pulse is rotated with winter cereals. *P. thornei* appears to be present mostly in the clay soils of the northern grain regions of Australia, while *P. neglectus* prefers the rather light soils of the southern part of the country (Thompson *et al.*, 2000). In nematicide trials, yield increases of 25–60% were observed (Thompson *et al.*, 2000), which gives an indication of the level of loss that can be incurred when the nematodes are present.

Management measures

Specific management practices have not been developed for lesion nematodes on chickpea. Most species of *Pratylenchus* have wide host ranges; therefore, control by rotation is problematic. This is especially true in rotations with winter cereals, which are often good hosts for the lesion nematode. However, rotation of cool-season with warm-season crops would be a satisfactory approach to control *P. thornei*.

Although chemical control is not an economically acceptable management measure, it has been demonstrated that split applications of aldicarb at 10 kg a.i./ha at sowing and after seed germination will control *P. thornei* and increase yield (Greco *et al.*, 1988b). Seed treatment with new-generation nematicides may be useful

for nematode management on this crop (see Chapter 23, this volume). Resistance to *P. thornei* was reported in several lines and accessions of cultivated (Ali and Ahmad, 2000) and wild chickpeas (Di Vito *et al.*, 2001).

Australian and international chickpea cultivars and germplasm accessions, and wild annual *Cicer* spp. in the primary and secondary gene pools, assessed for resistance to *P. thornei* and/or *P. neglectus* demonstrated that wild relatives from both the primary (*C. reticulatum* and *Cicer echinospermum*) and secondary (*C. bijugum*) gene pools of chickpea were generally more resistant than commercial chickpea cultivars to either *P. thornei* and/or *P. neglectus*. Further breeding could yield cultivars with high levels of resistance in many parts of the world where lesion nematodes are important in chickpea and also infect wheat production (Thompson *et al.*, 2011).

Highly heritable resistance to *P. thornei* was detected in recent Australian chickpea germplasm using an optimized glasshouse method and multi-environment trial analysis and demonstrated that further breeding and selection should provide incremental improvements in nematode resistance (Rodda *et al.*, 2016).

Rotylenchulus

R. reniformis has been found associated with chickpea in India (Rashid *et al.*, 1973; Ali, 1995) and in Ghana (Edwards, 1956). Another reniform nematode, *Rotylenchulus macrostoma*, occurs in chickpea fields in Syria, but it has never been found in the roots of this pulse. *R. reniformis* survives in the soil in the juvenile and adult male stages. Immature females penetrate the root and become established in the endodermis (Rebois *et al.*, 1975). The kidney-shaped females produce a gelatinous matrix that covers the female body and in which about 50 eggs are laid. Soil adhering to this matrix can often hamper detection of the female on the root surface.

Economic threshold level

Mahapatra and Padhi (1986) demonstrated in greenhouse tests that population densities of ≥ 0.5 nematodes/g of soil reduced plant growth,

and that growth reductions of 80% occurred at 10 nematodes/g of soil.

Management measures

Rotations designed to reduce nematode densities are difficult to develop because of the nematode's wide host range. The only acceptable recommendation is to avoid growing chickpea in heavily infested fields and to test local crops for non-host status before suggesting alternative cropping systems. However, in India, paddy rice reduces populations of several nematodes, including *R. reniformis* (Haidar *et al.*, 2001).

Other nematodes of chickpea

Several other nematode species have been found associated with chickpea (Ali, 1995). In South Australia, the 'oat race' of *D. dipsaci* is considered a severe problem on chickpea and pea. Young plants are very susceptible to the nematode, while adult plants are resistant (Thompson *et al.*, 2000). Species of *Amplimerlinius*, *Aorolaimus*, *Helicotylenchus*, *Merlinius*, *Criconemoides*, *Paratrophurus*, *Pratylenchoides*, *Tylenchus*, *Tylenchorhynchus* and *Zygotylenchus* were commonly found associated with chickpea in Mediterranean countries (Greco *et al.*, 1992; Di Vito *et al.*, 1994a,b; Castillo *et al.*, 1996). *Helicotylenchus indicus*, *Helicotylenchus sharafati*, *Hoplolaimus dimorphicus* (Mulk and Jairajpuri, 1974, 1975), *Tylencholaimus asymmetricus* (Khan and Ahmad, 1994), *Tylenchorhynchus vulgaris* (Gill and Swarup, 1977), *Tylenchorhynchus cicerus* (Kakar *et al.*, 1995) and many others (Ali, 1995; Ali and Sharma, 2003) were found associated with chickpea in India. Species of *Tylenchus*, *Scutellonema* and *Aphelenchoides* were observed in Sudan (El Tigani *et al.*, 1970), and *Tylenchorhynchus annulatus*, *Helicotylenchus digonicus* and *Hoplolaimus indicus* in Pakistan (Maqbool, 1986). With the exception of *H. indicus* and *T. vulgaris*, the pathogenicity of these nematodes has not been demonstrated. Sartaj *et al.* (1999) observed significant damage to chickpea caused by 500 *H. indicus* specimens per plant, and Gill and Swarup (1977) demonstrated that densities of *T. vulgaris* ranging from 10 to 20,000/500 g of soil caused increasing reductions in plant growth. Control measures have never been developed for these marginal pests.

Lentil

Lens culinaris, is a small-seeded legume that has been cultivated since ancient times in the Mediterranean region, and more recently in Asia and in the Americas. India with 34%, Canada with 19% and Turkey with 12% of total world production are the largest growers of lentil. The crop is also important in Syria, Bangladesh, Iran, Pakistan, China, Ethiopia, Morocco, Spain, Chile, the USA and Australia. Lentil is a winter crop normally rotated with cereals and cultivated from sea level up to more than 3000 m elevation. It is moderately resistant to low temperature and drought, but yields poorly in wet soils. Lentil is used mainly for human consumption in soup, roasted as a snack, and for baking flour. The straw has a high nutritional value and is commonly used as animal fodder.

Heterodera

H. ciceri is a major limiting factor affecting lentil production in north Syria and is the only cyst

nematode known to damage lentil in the field. The nematode causes severe stunting and yellowing of the crop (Fig. 9.7).

Economic threshold level

Lentil is less susceptible than chickpea to this cyst nematode. The tolerance limit (Greco *et al.*, 1988a) on lentil was 2.5 eggs/cm³ of soil compared with 1 egg/cm³ for chickpea. Yield losses of 20% occurred in fields infested with 20 eggs/cm³ of soil, but losses up to 50% occurred when population densities exceeded 64 eggs/cm³. Lentil produced on fields infested with *H. ciceri* also contained less protein. *H. ciceri* reproduction in the field was similar to that on chickpea at low population densities. Lower reproductive rates, however, were obtained at ≥ 2 eggs/cm³ of soil, due to lower numbers of new cysts produced and reduced number of eggs per cyst (Greco *et al.*, 1988a). Biological and chemical soil and seed treatment for nematode management have been studied with moderate effectiveness by Haseeb and Kumar, (2011), but are not economically viable as compared to using resistant varieties.



Fig. 9.7. Stunted and chlorotic lentil plants infested with *H. ciceri* in north Syria. (Photograph courtesy of N. Greco.)

Ditylenchus

D. dipsaci, the stem and bulb nematode, has been reported on lentil in Syria (Greco and Di Vito, 1987) and was isolated from the base of stems showing brownish necrotic lesions. Although the impact of the nematode on crop growth has not been measured, it can be assumed that *D. dipsaci* could damage lentil if late winters and early springs are cool and moisture levels are high.

Avoiding rotations with other host plants for the nematode, wider row spacing and proper weed control should be adequate to limit damage caused by the stem nematode. Augustin and Sikora (1989a) reported on the importance of weeds in Syria on population dynamics of the 'giant race' of *D. dipsaci* (now *D. gigas*).

Other nematodes of lentil

Among other nematodes occasionally found in the rhizosphere of lentil are *Helicotylenchus mucronatus* (Mulk and Jairajpuri, 1974) and *M. javanica* (Prakash, 1981) in India and *M. incognita* in Pakistan (Maqbool, 1986). An interaction between *M. javanica* and *F. oxysporum* f. sp. *lentis* on lentil has been observed leading to increased damage (Ali and Dwivedi, 2001). However, the root knot nematode species should not constitute a problem, because lentil is a winter crop and low temperatures are unfavourable for the development of these two species. Populations of *P. mediterraneus*, *P. neglectus*, *P. penetrans*, *P. pinguicaudatus*, *P. thornei* and *Pratylenchoides leiocauda*, from the Mediterranean basin, can infect lentil, but their impact on crop production has not been assessed (Di Vito *et al.*, 1994a,b, 2002a). *R. reniformis* is reported on lentil in India (Fazal *et al.*, 1995).

Mung Bean

Mung bean (*Phaseolus aureus*, syn. *Vigna radiata* var. *radiata*), also known as green or golden gram, probably originated in India. It is an annual, warm-temperature crop that can be planted in both the main growing season and as a mid-season crop. It is an important grain crop and is probably best known when used as a vegetable in

the form of bean sprouts. It is often rotated with rice, where it is planted directly into the stubble by broadcasting, or it is intercropped with cereals. Mung bean is tolerant to alkaline and saline growing conditions.

Meloidogyne

All four major species of root knot nematodes have been shown to parasitize mung bean. Species of *Meloidogyne* are a serious problem in India, Thailand, the Philippines and the USA (Bridge, 1981). *M. javanica* has been shown to cause damage to the crop in the Philippines (Castillo, 1975).

Prasad *et al.* (1971) evaluated field damage and noted that the nematode had a greater impact on grain formation than on pod setting. Root knot nematodes caused severe galling of the root system, chlorosis and stunting. *M. incognita* caused significant reductions in plant growth, nodulation and nitrogen content of the shoot and root (Hussaini and Seshadri, 1975; Inderjit Singh *et al.*, 1977).

Although no apparent differences in shoot growth were noticed after 2 months, when 14-day-old plants were inoculated with 0, 10, 25, 50 or 100 *M. javanica* egg masses (Catibog and Castillo, 1975), the severe root galling produced indicated that inoculation at planting would have resulted in greater losses. Losses of 28% were measured in a field infested with a mixed population of *M. incognita* and *R. reniformis* (Castillo *et al.*, 1977).

R. reniformis is considered to be a parasite of mung bean in a number of countries, but little is known about its economic importance on the crop.

Management measures

Standard crop rotations, especially those that include paddy rice, probably limit the degree of damage caused by nematodes on this crop. The extent to which root knot nematodes affect the crop in multiple cropping situations is unknown.

The use of nematicidal soil and seed treatment was shown to reduce populations of root knot and improve plant growth (Bhat *et al.*, 2012; Sitanshu *et al.*, 2012). Whereas soil treatment is not economically feasible under practical conditions, seed treatment with nematicides

might be practical. Although a number of breeding lines have been shown to be moderately resistant to *M. incognita* in India, cultivars with good agronomic characteristics are not available.

Cowpea

Cowpea (*Vigna unguiculata*) in the dry grain form is known as black-eyed pea, southern bean, China pea and marble pea, and in the green pod form as yard-long bean, asparagus bean, Bodi bean and snake bean. It is an annual plant with a great deal of varietal differences, including climbing, bushy prostrate and erect forms that probably originated in Africa or South-east Asia. Although the plant is used mainly for dried seeds, it is also used as a vegetable, pot herb and green manure. It is a hot-weather crop, well adapted to the semi-arid and hot humid growing regions. It is usually grown under rainfed conditions on well-drained soil (Kay, 1979). It is often intercropped with cereals, especially sorghum and millet, and can be planted without land preparation.

Meloidogyne

Root knot nematodes are serious pests of cowpea worldwide. *M. incognita* and *M. javanica* are the major species found on cowpea in most growing regions. Other important species are *M. arenaria* reported from Brazil, Cyprus, Nigeria and the USA; *Meloidogyne hapla* from Brazil; *Meloidogyne ethiopica* from Tanzania; *Meloidogyne africana* from East Africa; *Meloidogyne kikuyensis* from Kenya; and *Meloidogyne enterolobii* from Florida, USA, also attacking the cv. Iron Clay resistant to *M. incognita* (Brito *et al.*, 2003a,b).

Yield losses caused by *M. incognita* were estimated at 5–10% from cowpea fields in Georgia, USA (Toler *et al.*, 1963). Yield reduction in susceptible and resistant cowpea cultivars was 72% and 20%, respectively, in Venezuela (Crozzoli *et al.*, 1997, 1999), while in Nigeria, yield losses ranged between 20 and 50% (Caveness, 1973; Ogunfowora, 1976). In California, *M. javanica* and *M. incognita* are considered serious pests of cowpea (Thomason and McKinney, 1960).

Olowe (2004) found *M. incognita*, *M. javanica* and *M. arenaria* in single and mixed populations in cowpea in Nigeria. Robinson (1961) reported the common occurrence of *M. javanica* in Australia. *M. arenaria* present in soil taken from groundnut (peanut) fields caused severe damage to cowpea in Alabama, USA.

Symptoms

Symptoms induced by root knot nematodes include patches of stunted and yellowed plants (Figs 9.8 and 9.9). Severe root galling occurs at high nematode densities, (Fig. 9.10). Severe damage can lead to reduced numbers of leaves and buds.

Economic threshold level

In India, the threshold level determined in glass-house studies was 100 juveniles of *M. incognita*/500 g of sterilized soil, with significant yield reduction occurring at 2 juveniles/cm³ of soil (Sarmah and Sinha, 1995). *M. javanica* densities of 1000 and 10,000/500 g of soil caused growth reductions in pot tests (Gupta, 1979). In Venezuela, the tolerance limit of the susceptible cv. Manuare was 0.03 eggs and juveniles/cm³ of soil for *M. incognita* race 2, while the resistant cv. Ojito Negro tolerated up to 0.74 eggs and juveniles/cm³ of soil (Crozzoli *et al.*, 1997, 1999).

Disease complexes

The presence of high densities of *M. javanica* on a cowpea cultivar tolerant to *E. oxysporum* f. sp. *tracheiphilum* resulted in an increase in wilting when compared to a susceptible cultivar (Thomason *et al.*, 1959). Moreover, Roberts *et al.* (1995) observed increased wilting of a susceptible cowpea cultivar with the concomitant presence of *M. incognita* and the wilt fungus, but nematode infection did not increase the wilting of wilt-resistant genotypes. Interactions were also observed between *M. incognita* and *Macrophomina phaseolina* (Devi and Goswami, 1992) and *Rhizoctonia solani* (Kassab and Ali, 1996). Adekunle and Owa (2008) and Iheukwumere *et al.* (2009) reported increased infection of Cowpea aphid-borne mosaic virus and *M. incognita* in simultaneous and successive infections. Both pathogens significantly reduced all plant growth and yield



Fig. 9.8. Poor growth of cowpea infested with *Meloidogyne javanica* in Nigeria. (Photograph courtesy of C. Netcher.)



Fig. 9.9. Leaf chlorosis of cowpea due to *Meloidogyne incognita*. (Photograph courtesy of N. Greco.)



Fig. 9.10. Massive *Meloidogyne incognita* galling on cowpea in Nigeria. (Photograph courtesy of J. Bridge.)

parameters where nematode inoculation preceded that of the virus. In addition, Adekunle and Owa (2008) found that the concomitant inoculation of both pathogens resulted in a shortened life cycle of the nematode in comparison to inoculation with the nematode alone, indicating that the virus further compromised the plant defence system.

High densities of *M. incognita* have also been shown to lead to poor nodulation and decreased nitrogen levels in the plant (Sharma and Sethi, 1976a; Abedinia, 1978; Ali *et al.*, 1981). In these studies, root knot galls were found on nodules, and nodules were also produced on the surface of nematode galls. The symbiotic interrelationship was not affected at low population densities. Taha and Kassab (1980) reported that *M. javanica*, when inoculated simultaneously with *Rhizobium*, did not affect nodulation.

Management measures

Crop rotation can be an efficient means of controlling root knot nematodes in cowpea. Proper selection and placement of non-host crops and

resistant cultivars in rotation with susceptible cultivars can lead to nematode control and yield increases. The wide host range of the three major species of root knot nematodes associated with cowpea and the poorly understood host spectrum of most other *Meloidogyne* species requires careful crop selection and testing prior to the development of rotation schemes. Proper selection of non-hosts is also required because of the presence of races within the genus *Meloidogyne*.

Rotations with graminaceous crops or *Crotalaria* were recommended by da Ponte (1972). Populations of root knot nematodes decreased greatly in fallowed plots when *Crotalaria spectabilis* was grown as a weed-free cover crop as compared with the control (Rhoades, 1964). Mulching with cowpea foliage was also highly effective in suppressing populations (Rhoades and Forbes, 1986).

Egunjobi *et al.* (1986) showed that *M. javanica* populations were lower when cowpea and maize were intercropped, as opposed to a monoculture of each crop. The results suggested that this cropping system could be used for control of the nematode. Interplanting marigold with cowpea in a naturally infested field significantly reduced the populations of root knot nematodes and *Pratylenchus* spp. Plant growth parameters also improved in marigold-interplanted plots compared to sole cowpea plots (Olabiyi and Oyedunmade, 2007). Incorporating marigold or sunn hemp in *M. incognita*-infected cowpea fields suppresses *M. incognita* populations, thus reducing damage (Adekunle, 2011).

Castillo *et al.* (1976) reported that one crop of paddy rice was sufficient to reduce root knot nematode infestations effectively in succeeding susceptible legume crops. The reduction was greater after paddy rice than with rotations of non-host crops, probably due to the major impact of anaerobic conditions on nematode survival (see Chapter 23, this volume). The rotation of cowpea with winter crops, such as rye and narrow-leaved lupin, may decrease populations of *M. incognita* and *Pratylenchus* spp. (Wang *et al.*, 2002). Dukes *et al.* (1979) demonstrated that resistant cultivars were more effective than non-fumigant nematicides in reducing root knot damage.

Although fumigant and non-fumigant nematicides reduce root knot densities and can cause significant increases in yield, their use on

cowpea is not economical, unless cowpea is grown for the production of green pods used as vegetables.

The application of *Leucaena leucocephala* combined with cowpea seed treatment with *Paeclomyces lilacinus* reduced root knot incidence in India (Hasan, 2004). Cowpea plants grew better with *Trichoderma viride* and *Gliocladium virens* seed treatment at 10 g/kg seed compared to untreated plants (Verma *et al.*, 2009). The number of galls and egg masses were also reduced significantly compared to untreated plants. Egg hatch of *M. javanica* was severely inhibited with *Bacillus subtilis*, *Bacillus thuringiensis* and *Bacillus cereus* in *in vitro* studies, and when applied to infested soil, root damage was reduced followed by improved plant growth (Dawar *et al.*, 2008).

Resistance

There have been many studies in the past for evaluating breeding lines and cultivars for resistance, as outlined by Sikora *et al.* (2005). Thomason and McKinney (1960) reported that all 44 cowpea cultivars and plant introductions tested showed some resistance to *M. incognita*, but were moderately to highly susceptible to *M. javanica*. Cowpea cultivars having good agronomic traits and resistance to *M. incognita*, viruses, several fungal pathogens and insects have been released or identified. Among the most promising are the cvs. Texas Pinkeye, Purple Hull (Miller and Scheuring, 1994), Carolina Crowder, Better Snap, Tender Cream (Fery and Dukes, 1992, 1995b, 1996) and California Blackeye 27 (Ehlers *et al.*, 2000), all in the USA. Pampo and Otilia in Brazil (da Ponte *et al.*, 1993) and Ojito Negro in Venezuela (Crozzoli *et al.*, 1995). Wang and McSorley (2015) reported the cvs. Colossus, California Blackeye No 5, Iron Clay, Magnolia Blackeye, Mississippi Purple, Mississippi Silver, Tennessee Brown and Zippercream as poor or non-hosts for *M. incognita*, with some being resistant to other *Meloidogyne* species. These resistant cultivars can be rotated with susceptible crops for the management of *M. incognita* (Ogallo *et al.*, 1999). Cowpea is used as a cover crop in some areas of the USA, and resistant cultivars are available and recommended for use in management programmes (Wang and McSorley, 2015).

Multiple genes seem to control resistance to *M. incognita* in cowpea. Most of the cultivars

contain the resistant gene *RK*, as in the cultivar Mississippi Silver, but Fery and Dukes (1995a) have found that three resistant lines (US 566, US 567 and US 568) possess a gene that conditions the resistance that is allelic to the *RK* gene. Moreover, Roberts *et al.* (1996) state that the accession IT84S-2049, from Africa, contains a new dominant resistant gene (*RK*) conferring resistance to several populations of *M. incognita* and *M. javanica* including populations virulent to cultivars containing the *RK* gene. Olowe (2007) found resistance in four (ACC 64298 (New Era), IT845 2246-4, IT95k 207-22 and IT97k 819-180) cultivars out of 68 that were screened for resistance to root knot nematodes with a reproductive factor ranging between 0.5 and 0.8.

Heterodera

The cyst nematode *H. cajani* has been found associated with cowpea in a number of regions of India (Koshy and Swarup, 1971b), and has been detected on cowpea in Egypt (Aboul-Eid and Ghorab, 1974). The host range is limited to the Leguminosae or Pedaliaceae (Sharma and Swarup, 1984). Although the nematode seems to be widespread in India, crop loss assessment data are lacking (Luc, 1985). In a glasshouse study, an Egyptian population retarded the emergence of leaves and reduced the number of flowering buds, flowers, growing pods and yield (Aboul-Eid and Ghorab, 1974).

Economic threshold level

Shoot length was reduced in glasshouse experiments when the population density ranged between 10 and 20 juveniles/100 g of soil (Sharma and Sethi, 1975; Zaki and Bhatti, 1986). Both root and shoot length was reduced at nematode densities of 100 juveniles/100 g of soil (Sharma and Sethi, 1975).

Disease complexes

The nematode can complete its life cycle on nodular tissue and can reduce the number of *Rhizobium* nodules (Sharma and Sethi, 1975). Cowpea growth was not affected when *Rhizoctonia bataticola* was inoculated prior to, simultaneously with or after *H. cajani* in glasshouse tests (Walia and Gupta, 1986).

Management measures

The most effective management measure for cyst nematodes is rotation with non-host crops. Cowpea rotated with paddy rice may be less affected by the nematode because of the negative effect of flooding on nematode densities. Although nematicides have been shown to suppress nematode attack, in general they cannot be used economically on this crop.

Resistance

In India, screening demonstrated that the cv. Rituraj was highly resistant, the cvs. Bandel and Pusa Komal were resistant (Devi, 2001) and the cv. Barsati Mutant was tolerant to the nematode (Sharma and Sethi, 1976b). Four of ten commonly cultivated cowpea cultivars evaluated were found to be resistant to *H. cajani* (Pandey and Trivedi, 2006).

Other cyst nematodes of cowpea

Heterodera glycines and *H. schachtii* have been reported on cowpea but at present are of unknown economic importance on the crop. *Heterodera vigni* reported from cowpea is now recognized as a junior synonym of *H. cajani*. Nine cultivars of cowpea tested for susceptibility to *H. glycines* were resistant to the nematode (Epps, 1969a). Four of ten commonly cultivated cowpea cultivars evaluated were found to be resistant to *H. cajani* (Pandey and Trivedi, 2006).

Rotylenchulus

R. reniformis has been found associated with cowpea in India, Nigeria and the USA. Yield losses were detected when soil was treated with 1,3-dichloropropene (1,3-D) or ultra-high-frequency electromagnetic energy (Heald *et al.*, 1974). In India, avoidable yield loss from reniform nematode infestation was ascertained by applying carbofuran 3G at 2.0 kg a.i./ha in a naturally infested field in a study by Roy *et al.* (2008). Egg mass index and nematode population were reduced in the treated plots, while green pod yield of cowpea increased by 10.5–14.5%.

Races

Dasgupta and Seshadri (1971) divided the nematode populations into two races on their ability to parasitize cowpea, castor bean or cotton, with race A reproducing on all three hosts and race B only on cowpea. More recently, Rao and Ganguly (1996) added millet and mustard to the above list of test plants and found that of six Indian populations of the nematode reproduced on cowpea, castor bean and cotton, one did not reproduce on millet or mustard; one reproduced on millet but not mustard; one reproduced on mustard but not millet; and one reproduced on both millet and mustard.

Economic threshold level

The nematode reduced the emergence of seedlings by 7–9 days and seedling density by 6–11% at densities of 1 nematode/g of soil in glasshouse studies (Nanjappa *et al.*, 1978). A significant reduction in height and fresh shoot and root weights was observed in pot tests with 1000 juveniles per plant (Gupta and Yadav, 1980).

Management measures

The narrow host range of the nematode, especially race B, should allow excellent control with crop rotation. For example, nematode densities were suppressed when cowpea was grown intercropped with maize (Egunjobi *et al.*, 1986). Although breeding lines have been found with resistance to the nematode, commercial cultivars are not yet available (Thakar and Patel, 1984).

The combined effect of *Trichoderma harzianum* at 2 g/kg soil as soil application, and 30% concentration of lantana (*Lantana camara*) as seed soaking, proved better over carbofuran 3G in enhancing the growth parameters of cowpea and minimizing infection of *R. reniformis* (Patil *et al.*, 2013).

The application of carbofuran 3G at 2 kg a.i./ha increased the green pod yield of cowpea by 10.5–14.5%, with significant reduction in egg mass index and nematode population (Roy *et al.*, 2008). The application of neem cake at planting was comparable to seed treatment with carbofuran and carbosulfan in reducing the egg mass index and populations of reniform nematode on cowpea. A combination of the seed dressing with carbosulfan with neem cake at planting provided

significantly reduced nematode populations and egg mass index, accompanied by a significant increase in plant growth and yield parameters of cowpea (Mukhopadhyay and Roy, 2007).

Other nematodes of cowpea

Hoplolaimus seinhorsti, an endoparasitic nematode, was shown to cause severe damage to cowpea in Nigeria. The nematode induced marked necrosis in both the lateral and secondary lateral roots in field plot studies in Nigeria (Bridge, 1973). After 9 weeks, most of the lateral feeder roots were rotted or missing. The number of nematodes increased to a maximum of 1110/root system after 5 weeks. de Siqueira and Inomoto (2008) reported the pathogenicity of *Pratylenchus brachyurus* on six cowpea cultivars in Brazil. They observed that the most susceptible cultivars were stunted and exhibited irregular root lesions or a large area of decayed tissue.

Haricot or Common Bean

Haricot (*P. vulgaris*), also known as common, navy, white pea, pea, French, kidney, string, salad, runner, bush, pole and turtle bean, originated in Mexico between 2300 and 4000 BC. It is the most widely cultivated food legume (Table 9.1 and 9.2), with approximately 30.1 Mha in production in 2014. Among the food legumes, *P. vulgaris* is the most uniformly distributed crop in the world and the main food legume in the Americas, where it is of great agricultural importance, especially in Brazil, Mexico and the USA. In Asia, haricot beans are cultivated extensively in India, with 36% of the world hectareage. Extensive plantings also exist in China, Indonesia, Iran, Myanmar (Burma), North Korea, Pakistan, Thailand, Turkey and Vietnam. In Africa, the main producers are Burundi, Cameroon, Congo, Ethiopia, Rwanda, Tanzania and Uganda. In Europe, with only 2% of world acreage, this pulse is only of importance in Albania, Belarus, Greece, Italy, Moldova, Poland, Romania, Spain, Ukraine and the former Yugoslavia. Nearly all countries in the tropics and subtropics produce *P. vulgaris* for dried grains, which are eaten whole or mashed,

mainly in soup. In addition to dried grain, the crop is used for fresh green seeds, whole pods, or are canned or frozen. In several countries, beans are also cultivated in glasshouses for the high-value fresh vegetable market.

Phaseolus species are sensitive to low temperature; therefore, in the subtropics, they are cultivated during the warm seasons and sown early in the spring, or in summer after the winter crop. The crop is therefore infected with many nematode species that have higher temperature optimums. The crop is grown as a sole crop, semi-climbing and as a climbing bean in relay systems with maize. Beans are often grown intercropped with maize.

Meloidogyne

M. incognita, *M. javanica* and *M. arenaria* appear to be the most common root knot nematode species of haricot beans and have been reported to cause damage in the Americas, Africa and Asia. There is probably no country in the tropics and subtropics in which beans are not affected by root knot nematodes. *M. enterolobii* has been reported to damage haricot bean in Florida, USA (Brito *et al.*, 2003b). This nematode is probably more widespread than is thought in tropical America, South Africa and West African countries (Brito *et al.*, 2003a). Moreover, *Meloidogyne chitwoodi* and *M. hapla* damage the crop in northern USA (Hafez and Sundararaj, 1999), and *Meloidogyne brasiliensis* has potential to do so in Brazil (Charchar and Eisenback, 2002).

Symptoms

Symptoms of root knot nematode attack are intense leaf chlorosis and stunting (Fig. 9.11). However, gall size on the roots of *Phaseolus* spp. is variable, and may be nearly undetectable (Blazey *et al.*, 1964). In the latter case, the only visible symptom on the roots is the presence of large egg masses. However, severe galling was observed in Brazil and Chile (Lordello and de Oliveira Santos, 1960). Due to the large number of types and cultivars of haricot bean and to the presence of root knot races (see Chapter 10, this volume), the intensity of damage caused by *Meloidogyne* spp. varies greatly.



Fig. 9.11. Leaf chlorosis and stunting of haricot bean due to *Meloidogyne* in Niger. (Photograph courtesy of R. Sikora.)

Disease complexes

M. incognita reduced the number and nitrogen-fixing efficiency of bacterial nodules on the roots of haricot bean (Singh and Reddy, 1981; Mohanty *et al.*, 2001), and has been shown to increase the severity of *M. phaseolina* (Al-Hazmi, 1985). The synchronized inoculation of *M. incognita* and *R. solani* increased the index of *Rhizoctonia* root rot and the number of root galls and suppressed plant growth, compared to controls. When nematode inoculation preceded the fungus, plant growth was severely suppressed and roots were highly damaged and rotted (Al-Hazmi and Al-Nadary, 2015). Hutton *et al.* (1972) and France and Abawi (1995) detected increased wilting by *Fusarium solani* f. sp. *phaseoli* on beans attacked by *M. arenaria*, *M. javanica* and *M. incognita*, and found that resistance to the fungus could be lost in the presence of *M. incognita* infection. Extreme fungal root rotting is often associated with root knot damage (Fig. 9.12).

Economic threshold level

The extent of yield loss caused by *Meloidogyne* spp. to haricot bean has not been assessed under field conditions. The information available was derived from yields obtained in nematicide trials or pot experiments. Sharma (1981) observed significant growth reduction in soil infested with *M. javanica* at 1 egg/g of soil, and a reduction of 82% at 10 eggs/g soil in glasshouse experiments. In pot experiments, Crozzoli *et al.* (1997) found tolerance limits of three haricot bean cultivars to *M. incognita* of 0.02–0.03 eggs/cm³ of soil,

and that yield of green pods was reduced to 35–53% in soil infested with 4 eggs/cm³ of soil.

Management measures

Abiotic stress caused by adverse environmental factors and interrelationships between root knot nematodes and other soil-borne pathogens are responsible for severe damage under field conditions (Fig. 9.12). Planting time certainly plays an important role on the amount of yield loss. Most species of *Meloidogyne* found in the tropics and subtropics would be unable to invade bean roots if the crop was sown at the end of winter or early in the spring, when soil temperatures would be below 15°C. Escape from early root penetration would give the plant an advantage over the nematode in the early stages of plant growth. Yield would increase because the larger root system could withstand the damage caused by nematode invasion later in the season. Moreover, these beans would be harvested by the end of spring or early in summer, thus limiting the number of nematode generations produced (often to only one) and overall population densities. Sowing bean late in spring or in summer would result in early nematode infection, the development of multiple generations, severe root damage and high nematode populations in infested soil.

Multispectrum fumigants such as Vapam, Telone C-17, Telone C-35 and Pic Clor 60 are often used as row treatments to reduce losses from both nematode and fungal pathogens in bean and pea crops in the southern part of the USA, where heavy nematode populations cause severe damage in sandy soils (Noling, 2015).



Fig. 9.12. *Meloidogyne incognita* galling and severe fungal rotting of haricot bean roots in the Philippines due to the interaction with soil root rotting fungi. (Photograph courtesy of R. Sikora.)

Haricot beans have relatively short growing seasons and, therefore, reduced rates of nematocides may be sufficient to give control and reduce possible environmental impact. The use of systemic nematocides in fresh vegetable production must be monitored closely, because of the relatively short growing season for these crops.

Resistance

The breeding lines B-3864 (Fassuliotis *et al.*, 1967) and B-4175 (Wyatt *et al.*, 1980), both resistant to *M. incognita*, were derived from the Mexican line PI 165426. Further selection enabled Wyatt *et al.* (1983) to release the cv. Nemasnap, the first bush snap bean cultivar resistant to *M. incognita*. Moreover, the cvs. Alabama N1, Carioca, Manoa Wonder and Riotibagi were found to be resistant to one or more species of root knot nematodes. More cultivars resistant to *M. incognita* are reported by Blazey *et al.* (1964). In Brazil, Ribeiro and Ferraz (1983) tested 49 cultivars and lines and found that 37-R, Honduras-35, 51051 and Rajado Ag. 496 could be considered resistant to *M. javanica*, although data were variable. In Kenya, the cvs. Kahuti, Red Haricot, Rono, Saginaw and Kiburn were resistant to local populations of *M. incognita* and *M. javanica* (Ngundo, 1977). In Turkey, 13 genotypes were found to be resistant to *M. incognita* among the 87 evaluated and were suggested for use as parental material for root knot resistance breeding programmes (Bozbuga *et al.*, 2015). The resistance to *M. incognita* in haricot bean was considered to be linked to two independent genes

in the cvs. Springwater Half Runner and Wingard Wonder, according to Blazey *et al.* (1964), and to three pairs of recessive genes, according to Hartman (1971), in the cv. Alabama No 1. Omwega *et al.* (1990a) conversely found that a single dominant gene (*Me1*) was responsible for resistance to *M. javanica*, *M. incognita* race 1 and *M. arenaria* race 1 in bean lines derived from the landraces G2618 and G1805. However, the reaction of the known resistant lines to different *Meloidogyne* species, populations or races may vary. Moreover, different sources of resistance are not equally heat stable, and heat stability also differs with nematode species and race (Omwega *et al.*, 1990b; Mullin *et al.*, 1991; Sydenham *et al.*, 1997). Most of the resistant germplasm lines are available at Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. However, before undertaking a breeding programme, it is suggested that the most suitable resistance source is selected on the basis of its reaction to local populations of root knot nematodes and environmental conditions.

Assuming that the mentioned resistant cultivars have good agronomic attributes and are suitable to local climates, they should be integrated into management systems in areas infested with root knot nematodes, taking into consideration that resistance management requires rotating with different sources of resistance and/or periodically with susceptible cultivars to prevent the formation of resistance-breaking *Meloidogyne* races.

Heterodera

The soybean cyst nematode *H. glycines*, besides infecting soybean, also attacks *Phaseolus* spp. This is important because *Phaseolus* beans are often rotated with soybean. Crop loss due to *H. glycines* infestations on haricot beans have been reported mainly in the USA (Noel, 1992).

Symptoms

Nematode infection is similar to that observed on soybean. In glasshouse tests, haricot bean was less susceptible to *H. glycines* than soybean (Abawi and Jacobsen, 1984). Data on yield loss incurred in the field are lacking. The level of invasion and reproduction of *H. glycines* on haricot

bean is similar to or larger than that encountered on soybean (Abawi and Jacobsen, 1984; Poromarto and Nelson, 2009).

H. glycines must be considered a potential problem on haricot bean in areas where the nematode occurs, especially if the crop is rotated with soybean or other susceptible host crops. Abawi and Jacobsen (1984) postulated that because of the larger root size of haricot bean compared with that of soybean, the reproduction rates of *H. glycines* on the former could potentially be greater under field conditions, and thus lead to larger soil population densities.

Management measures

The control measures devised for control of *H. glycines* on soybean should also be used on haricot beans. There seems to be large variation in cultivar susceptibility to the nematode. The cvs. Kentucky Wonder Pole and Kentucky Wonder Improved Rust Resistant are resistant to *H. glycines* (Melton *et al.*, 1985) and should be used to avoid yield losses and reduce nematode population densities when *H. glycines* is present in the field.

Rotylenchulus

The reniform nematode, *R. reniformis*, also damages haricot bean, especially, but not only, in southern USA and tropical American countries (Tarte, 1971). This nematode also reduces *Rhizobium* root nodulation and seed protein content, and increases the severity of the fungus *E. solani*. Investigations on yield loss and control have been reported (McSorley, 1980; McSorley *et al.*, 1981; McSorley and Pohronenzy, 1984). Nematode threshold levels, however, have not been determined. Rotations with cotton should be avoided, because pre-plant soil populations of *R. reniformis* following cotton were shown to be ten times higher than following grain sorghum rotations (Thames and Heald, 1974). Information on resistant cultivars is scarce.

Pratylenchus

Several lesion nematodes have been reported on haricot bean, causing extensive root necrosis

and yield reduction. Among them, *Pratylenchus scribneri* (Thomason *et al.*, 1976) and *P. penetrans* (Elliot and Bird, 1985) have been shown to reduce plant growth when soil populations exceed 0.5 nematodes/cm³ of soil. The cvs. Saginaw, Gratiot and Kentwood were tolerant to *P. penetrans*. It should be noted that *P. penetrans* reduced levels of arbuscular mycorrhiza (*Glomus fasciculatum*). The latter is important in phosphorus uptake by the root system. Although *P. penetrans* reproduction was not affected by mycorrhiza, the presence of the fungus symbiont reduced the severity of nematode damage. This indicates that mycorrhizal fungi are important in regulating nematode populations in haricot bean (Elliot *et al.*, 1984).

The cosmopolitan species *P. neglectus*, *Pratylenchus alleni*, *P. brachyurus* and *P. thornei* infect haricot bean. Moreover, populations of *P. zeae* from Brazil, Malawi and Mozambique, *P. pinguicaudatus* from North Africa, and the banana lesion nematode *Pratylenchus goodeyi* from Uganda were found to reproduce on haricot bean. Their importance in crop production is unknown.

Means of management suggested for root lesion nematodes on other crops should work satisfactorily on haricot bean.

Other nematodes of haricot bean

The false root knot nematode *Nacobbus aberrans*, another sedentary endoparasitic nematode, is found in the Americas, and populations of the nematode from the states Puebla, Guanajato, Zacatecas and San Luis Potosi in Mexico damage haricot bean, with yield losses up to 36% having been reported (Lehman, 1985; Toledo *et al.*, 1993; Manzanilla-Lopez *et al.*, 2002). Infected roots show large galls similar to those of *Meloidogyne* spp. Therefore, close observation is required for correct diagnosis. *N. aberrans* seems to be less pathogenic than root knot. One generation requires 36 days at 25°C. The nematode has a wide host range, including sugar beet, tomato, potato, pepper, and many cruciferous plants and a variety of weeds. The wide host range complicates the development of effective rotation systems for control purposes. The nematode reproduces well in a number of different soil types and damage is not restricted to sandy soils, as is the case with most root knot species. Nematode populations

from different areas may have diverse host ranges, indicating the possible existence of races or pathotypes. Populations of *N. aberrans* damaging haricot bean in Mexico only infect haricot bean and chilli pepper, and are classified as belonging to 'the bean group' (Manzanilla-Lopez *et al.*, 2002). Moreover, the cvs. Amarillo Calpan, Bayo Mecentral, Negro San Luis and Rio Grande are resistant to Mexican populations of the nematode (I. Cid del Prado-Vera, Mexico, 2003, personal communication).

Foliar damage caused by *Aphelenchoides ritzemabosi* was sometimes observed on haricot bean following lucerne in Wyoming, USA. The nematode is common in lucerne fields, along with *D. dipsaci*. It persisted up to 27 months in dried bean leaves, thus facilitating its persistence. However, this nematode is not considered to cause economic loss unless environmental conditions are suitable (Franc *et al.*, 1996). *Belonolaimus longicaudatus*, *Belonolaimus gracilis*, *Hoplolaimus galeatus*, *Zygotylenchus guevarai*, *Helicotylenchus dihystra*, *Tylenchorhynchus acutus* and *Dolichodorus heterocephalus* have also been reported from haricot bean. The potato rot nematode *Ditylenchus destructor* (MacGuidin and Slack, 1991) and *Hemicycliophora poranga* (Chitambar, 1993) have the potential to damage haricot bean in the USA. Yield increases have been obtained following the application of nematicides in infested fields. Studies on their threshold levels and the exact extent of yield loss associated with these nematodes have not been conducted. These nematodes often occur concomitantly with economically important species, e.g. *H. glycines*, *R. reniformis* and species of *Meloidogyne* and *Pratylenchus*. Nematicides when suggested for the control of the latter are usually effective against nematodes of lesser importance in the same field.

Pea

Pea (*P. sativum*), also known as garden pea, English, Chinese or snow pea, is a food legume used as both a dried grain and a fresh vegetable. Pea was originally cultivated for grain, and only in the 16th century did the use of fresh seeds become popular. In the past few decades, pea has probably become the most common frozen vegetable in Europe and the USA. Europe accounts for 33% of the world pea production, followed by

China with 12% and India 11%. Small amounts are grown in Burundi, Ethiopia, the USA, Peru, Pakistan, Denmark, France, Hungary, the UK and Australia. Only 0.8 Mt are devoted to the production of green peas for the frozen food industry. Pea straw is also used for livestock feeding.

Heterodera

The cyst nematodes *H. goettingiana*, *H. trifolii* (Mulvey and Anderson, 1974) and *H. ciceri* (Greco *et al.*, 1986b) reproduce well on garden pea. No damage by the latter two species has been reported on pea in the subtropical regions of the Mediterranean, where both species occur. In Mongolia, a population of *H. glycines* was found to reproduce on pea (Zhang, 1995). The most noxious cyst nematode affecting pea is *H. goettingiana*. This cyst nematode is widespread in Europe and the Mediterranean basin. In 1992, severe infestations of pea crops by *H. goettingiana* were observed in the state of Washington in the USA (Handoo *et al.*, 1994).

Infested pea fields show patches in which plants are stunted, chlorotic, produce fewer flowers and small and often empty pods. Symptoms of nematode infestations are very evident at flowering. Heavily infected plants have large numbers of swollen females on the surface of roots (Fig. 9.13). The root systems are reduced in size and exhibit poor nodulation. Additional applications of fertilizer may not lessen damage.



Fig. 9.13. Roots of peas heavily infested with white females of *Heterodera goettingiana*. (Photograph courtesy of N. Greco.)

Damage is amplified by an interrelationship of *H. goettingiana* with the soil-borne fungus *F. oxysporum* f. sp. *pisi* (Garofalo, 1964). In dry areas, pea suffers greatly from drought, due to the reduced size and efficiency of the root system. Senescence also tends to occur earlier.

Economic threshold level

The extent of damage caused by the nematode varies with cultivar and environmental conditions. However, Greco *et al.* (1991) reported a tolerance limit of garden pea to *H. goettingiana* of 0.5 eggs/cm³ of soil, with 20–50% yield losses expected at between 3 and 8 eggs/cm³ of soil. Complete crop failure occurs at densities of ≥ 32 eggs/cm³ of soil.

Other hosts

H. goettingiana reproduces well on garden pea (*P. sativum*), field pea (*Pisum arvense*), broad bean (*V. faba*), vetch (*Vicia* spp.) and grass pea (*L. sativus*). Reproduction on other cultivated leguminous species is negligible. Several wild species of *Vicia* and *Lathyrus* (Jones, 1950; Winslow, 1954) are also hosts and are responsible for maintaining high nematode soil densities even in the absence of host crops.

Biology

The time required by juveniles to reach the adult stage is influenced strongly by temperature and can take 7 weeks in winter and only 2 weeks in spring (Greco *et al.*, 1986a). *H. goettingiana*, having a minimum temperature for development of 4.4°C, can penetrate and develop on pea during the winter season (Beane and Perry, 1984). On garden pea sown in mid-autumn, females are formed by the end of autumn or in early winter. In this season, soil temperature is below 15°C, and the females form egg masses containing 100–150 eggs. When peas are sown in late autumn, females are usually observed in the spring when soil temperature start to exceed 15°C and sufficient soil moisture is available. At these low temperatures, egg masses will usually not be formed behind the female cyst, or they will be small and empty of eggs. Eggs in egg masses hatch promptly, however, at temperatures between 15 and 20°C, when adequate soil moisture is available. Egg hatch is suppressed at 25°C

and therefore no root invasion would occur during the warm season. In England, one generation per year was reported on garden pea and two on broad bean (Jones, 1950). In the subtropical climate of the Mediterranean region, only one generation is completed on pea sown from late autumn onwards, but two to three generations if pea is sown in early autumn. In the latter case, egg masses could be produced and a high reproduction rate expected (Greco *et al.*, 1986a).

Management measures

Management of *H. goettingiana* varies with crop type. Cultivation of early pea for green pod production usually gives high return, and therefore the use of nematicides is economical. Soil solarization could be an alternative method for cyst nematode control on high-value crops (Greco *et al.*, 1985). Mulching irrigated soil with thin (30–50 µm) polyethylene sheets for 4–8 weeks can reduce *H. goettingiana* in regions with sufficient solar energy, assuming that the field can remain free of crops for the required time. However, solarization and non-fumigant nematicides are usually less effective than fumigants.

None of the above methods is economically acceptable when garden peas are grown for the production of dried grain. Rotating pea with non-host crops for a 3- to 6-year period will reduce nematode densities to non-damaging levels, assuming an annual population decline of 50% (Di Vito and Greco, 1986).

Meloidogyne

Garden pea is a good host for root knot nematodes, even though reports on infestations are limited. *M. incognita* was reported on pea in India (Reddy, 1985). There is little doubt that this and the other warm-season root knot nematodes can be important parasites of peas in the tropics. *M. artiellia* has the potential to damage pea in the Mediterranean area, and *M. brasiliensis* has been found infesting pea in Brazil (Charchar and Eisenback, 2002). In the subtropics, pea is grown mostly as a winter crop, and therefore damage caused by root knot nematodes is negligible, unless pea is sown early in autumn after a summer host crop, in which case pea growth would be

reduced at an early stage of development. Above-ground symptoms of nematode attack are similar to those outlined for *H. goettingiana*. The roots exhibit large galls and reduction in size, and *Rhizobia* nodulation is also reduced in the presence of nematode infection. An interaction between *M. incognita* and *E. oxysporum* f. sp. *pisi* on pea has been demonstrated by Siddiqui *et al.* (1999). The tolerance limit of pea to *M. incognita* was about 0.5 eggs/g of soil (Siddiqui *et al.*, 1995) and probably less to *M. javanica*.

Peas escape nematode attack in the subtropics, when sowing is postponed to mid-autumn, or when temperatures drop below 15°C. The use of soil fumigant row treatments are used in Florida on sandy soils for the control of root knot and fungal soil-borne pathogens, as well as other nematodes on peas where damage can be severe (Noling, 2015). Seed treatment with nematicides and neem-based products has been shown to increase yield (Mani and Sethi, 1984; Mojumder *et al.*, 2002; Pankaj *et al.*, 2006). A number of studies with biological control agents have shown impact on root knot nematodes, but they are not considered economically acceptable for nematode control. Management of root knot should be carried out in an integrated systems approach, taking advantage of all tools available to the grower (Noling, 2015). Currently used management practices include: crop rotation of non-host or less susceptible crops; resistant varieties; beneficial cultural practices; as well as pre-plant nematicide treatments. Noling (2015) recommended integrated these practices in the 'off-season' cropping sequence.

Multispectrum fumigants such as Vapam, Telone C-17, Telone C-35 and Pic Clor 60 are often used as row treatments to reduce losses from both nematode and fungal diseases. Relatively low label rates are sometimes adequate for nematode and disease suppression for snap beans because of their short growing season (45–60 days), which reduces the time available for pest reproduction.

chlorosis. These symptoms can be confused with those produced by other nematodes and diseases. *D. dipsaci* damages epidermal, cortical parenchyma and external phloem tissue, thereby adversely affecting translocation processes. In Australia, severe damage is caused by *D. dipsaci* on pea at the seedling stage, and a 30% reduction in seedling emergence has been observed (Thompson *et al.*, 2000). Infected pods are distorted and contain few seeds, which in turn may also be infected. It is not known whether the nematode can survive for an extended period within grains, as is often observed on other crops. Infestation of pea seed is significantly less than that reported in broad bean (Knuth, 1993).

In the subtropics, attacks of *D. dipsaci* are more severe on garden pea sown in autumn, and symptoms become more obvious throughout late winter and early spring. The same management measures suggested for this nematode on broad bean should also be adopted on pea. The root lesion nematodes *P. crenatus* and *P. penetrans* have been found in association with pea decline. Mediterranean populations of *P. neglectus*, *P. penetrans*, *P. pinguicaudatus* and *P. thornei* have potential to damage pea (Di Vito *et al.*, 2002a), whereas pea appears to be resistant or tolerant to Australian populations of *P. thornei* and *P. neglectus* (Thompson *et al.*, 2000). Symptoms caused by these nematodes are similar to those observed on other crops. *Pratylenchus* spp. are also known to break down plant resistance to *Fusarium* wilt (Oyekan and Mitchell, 1971). *R. reniformis* is found worldwide and damages pea, especially in India, where a tolerance limit of 0.1 nematodes/g of soil is estimated (Vats and Dalal, 1998). The nematode reduces *Rhizobium* nodulation and may interact with *E. oxysporum* f. sp. *pisi* (Vats and Dalal, 1997). In Brazil, *H. dihystra* is considered a severe constraint of wheat and pea (Sharma *et al.*, 1993). *Meloidogyne pisi* n. sp. has been described from specimens obtained from roots of pea cv. Mikado in Brasilia, Brazil (Charchar *et al.*, 2008).

Other nematodes of garden pea

D. dipsaci damages garden pea in several countries (Hooper, 1972; Thompson *et al.*, 2000). Infected plants show extensive brownish and necrotic lesions on the stems (Fig. 9.13) and leaf

Pigeonpea

Pigeonpea (*Cajanus cajan*), also known as red gram, Congo pea and no-eyes pea, originated in Africa around 2000 BC. Pigeonpea is a woody, short-lived, perennial shrub that reaches a height

of 3.5 m. It is grown in the tropics and subtropics and is very common in India, where over 80% of the world crop is grown and consumed. This drought-resistant crop is often intercropped with cereals in India and Africa, especially in semi-arid regions. The crop, which is usually planted as an annual and grown for dried grain, is used for dhal (decorticated split seed) in a variety of foods. In other countries, the green seeds are eaten as a substitute for, or in preference to, green peas. A large number of plant parasitic nematode species have been found associated with pigeonpea on a worldwide basis (Sharma, 1985). The vast majority are of limited economic importance. However, recent research work has shown that significant yield loss is exerted on the crop by some species of plant parasitic nematodes.

Heterodera

The cyst nematode *H. cajani* described by Koshy (1967) was first recorded on pigeonpea in India by Swarup *et al.* (1964). The nematode was subsequently reported attacking the crop in a number of states in India (Sharma and Swarup, 1984). The exact distribution and frequency of occurrence within the country, however, have not been determined. The nematode was detected in only 7 out of 471 fields examined by Koshy and Swarup (1971a), and more recently has been detected in a large number of experimental fields in central India. The nematode is more prevalent on vertisol rather than alfisol soils. Sharma *et al.* (1992) have reviewed the

importance, biology and control of nematodes infecting pigeonpea.

Symptoms

In the field, yellowing and stunting have been observed; the former varies with plant genotype (Fig. 9.14). In glasshouse tests, plants infected with 1000 or 5000 juveniles/500 cm³ of sterilized soil were stunted with smaller internodes and leaves.

Other hosts

More than 105 plant species belonging to 58 genera in the families Leguminosae and Pedaliaceae are known hosts of *H. cajani* (Koshy and Swarup, 1972). Important hosts are chickpea, horse gram, hyacinth bean, soybean, tepary bean, moth bean and a number of species in the genera *Phaseolus* and *Vicia*.

Economic threshold level

Field densities have been shown to range from two to 130 cysts/500 cm³ of soil. The highest numbers were detected on perennial plants or in fields cropped successively for 3–4 years (S.B. Sharma, India, 1988, personal communication). Plants associated with high cyst densities growing in vertisol soils were stunted and frequently chlorotic. Symptoms of damage seemed to be more prevalent in the Kharif crop planted in the autumn. Initial populations of five juveniles/100 cm³ of soil were found to affect plant growth. Zaki and Bhatti (1986) reported that 100 juveniles/kg



Fig. 9.14. Stunted and chlorotic pigeonpea plants infested with *Heterodera cajani* in India. (Photograph courtesy of S.B. Sharma.)

of soil caused significant reductions in growth in pot trials. Nematicide treatment led to grain yield increases of 20–25% over controls (Sharma *et al.*, 1993).

Biology

At a soil temperature of 29°C, the nematode completes one generation in 16 days (Koshy and Swarup, 1971a). Optimum temperature for emergence is 28°C, with distinct reductions in emergence at 25°C (Sharma and Swarup, 1984). In India, the largest number of juveniles emerged between August and October. An initial density of 1 juvenile/cm³ of soil caused a 14–24% reduction in plant growth. The tolerance limit in the field was estimated at 2.6 eggs and juveniles/cm³ of soil at sowing time (Sharma *et al.*, 1993).

Disease complexes

Wilt caused by *Fusarium udum* increased significantly when combined with *H. cajani* in greenhouse tests. The pigeonpea lines used, however, reacted differently to the nematode–fungus combination. In one instance, the pathogenic effects of the nematode on plant growth were negated in the presence of the fungus (S.B. Sharma, India, 1988, personal communication).

Although *H. cajani* females have also been observed attached to *Rhizobium* nodules, nothing is known about the effects of the interrelationship on plant health.

Management measures

Strategies for control of the nematode should place stress on crop rotation and resistance if commercially available. Rotations with cereal crops, especially millet, probably limit nematode damage. *Echinochloa colona* (barnyard millet), *Paspalum scorbiculatum* (Kodo millet), *Setaria italica* (Italian millet), *Chionachne* spp., *Trilobachne* spp. and *Zea mexicana* (teosinte) were shown to be non-hosts (Sharma and Swarup, 1984) and could be used effectively in a crop rotation sequence.

Although soil application of nematicides has been used to demonstrate the impact of nematodes on yield, in most cases, they are not considered to be economically feasible. However, Zaki and Bhatti (1986) used seed treatment with nematicides to reduce nematode densities. This approach could be considered as a potential alternative control option with some of the more

recently labelled compounds (see chapter 23, this volume).

Resistance

Many of the pigeonpea types and genotypes grown are unimproved landraces. This germplasm should serve as a basis for the development of nematode-resistant cultivars with good agronomic characteristics. A number of lines have been reported to be resistant to the nematode; however, retesting has not always substantiated the results (Devi, 1998). Variation in testing techniques and in reporting the degree of resistance must be monitored more closely to avoid improper designation of the level of resistance.

Meloidogyne

M. javanica was found on pigeonpea in Puerto Rico (Ayala, 1962a), Brazil (Lordello and Arruda, 1956) and Malawi (Reddy *et al.*, 1993). Pigeonpea was shown to be highly susceptible to a population of *M. arenaria* taken from groundnut fields in Alabama, USA (Rodriguez-Kabana and Ingram, 1978). The nematode causes significant amounts of galling on the root system, leading to reduced growth and overall yield. Plant growth was reduced significantly in pot tests at initial densities of 100 juveniles/500 g of soil (Pathak *et al.*, 1985). Salam and Khan (1986) reported that *M. javanica* caused increased wilt in plants affected by *E. oxysporum* f. sp. *udum*, and Dwivedi *et al.* (1992) demonstrated the same for *M. incognita*.

Field trials with nematicides where galling was reduced between 53 and 61% demonstrated a 14.2% avoidable yield loss due to a mixed population of *M. incognita* and *M. javanica* (Patel and Patel, 1993). Seed treatment with neem-based products reduced nematode infection (Dibakar and Mishra, 2000).

Many accessions and cultivars have been shown to have resistance to *M. incognita* (Wani and Alam, 1995; Suhail *et al.*, 2001). A number of breeding lines have been shown to be highly resistant to both *M. incognita* and *M. javanica*, but are susceptible to *E. udum* (Patel *et al.*, 1987). Acosta *et al.* (1986) reported that all cultivars tested were susceptible to *M. javanica*. Siddiqui *et al.* (1991) detected resistance to *M. arenaria* race 2, as well as to *M. incognita* and *M. javanica*, but not to *M. arenaria* race 1.

Rotylenchulus

Linford and Oliveira (1940) in Hawaii were the first to report *R. reniformis* on pigeonpea. It has since been reported attacking the crop in Puerto Rico, Jamaica and India. The nematode causes yellowing of new leaves, progressive dieback of twigs and main stems, and premature death of many plants in Jamaica (Hutton and Hammerton, 1975). Galls are not produced on the root system as with root knot nematode. However, the females embedded in their egg masses on the surface of the root are diagnostic for infection. Although the root system was reduced in size, extensive necrosis was not observed. Root death seemed to be caused by excessive infection of the root tip (Ayala, 1962b). Jain and Sharma (1996) showed that the nematode increased the intensity of *E. udum* wilt on the wilt-resistant cv. ICPL 270 in the fields.

Thakar and Yadav (1985a) reported significant reductions in plant weight at 1000 or 10,000 nematodes/700 g of soil in pot tests on susceptible or resistant cultivars, respectively. Suppression of growth was also detected at densities of 100 nematodes/500 g of soil (Pathak *et al.*, 1985).

The nematode also reproduced on *Rhizobium* nodules (Ayala, 1962c). In a glasshouse experiment, race A caused marked reductions in total plant fresh weight after 30 days at a density of 142 nematodes/100 g of soil (Thakar and Yadav, 1985a).

Pigeonpea lines have been shown to be moderately resistant to the nematode (Thakar and Yadav, 1985b; Patel *et al.*, 1987; Suhail *et al.*, 2001). Resistance was reported in the accessions Pusa-33, 78, 84 and 85 in India (Ahmad, 1992). Field testing at ICRISAT showed that some germplasm had tolerance to the nematode (Sharma *et al.*, 2000). In a study to evaluate genotypes for resistance to *R. reniformis* in Brazil, all ten genotypes tested were susceptible to the nematode (Araújo *et al.*, 2010).

Other nematodes of pigeonpea

Pigeonpea is used in Brazil as a rotation crop for soil improvement in sugarcane, where it is highly susceptible to *P. zeae*. Souto *et al.* (2011) identified 13 lines, as well as the cultivar Fava

Larga, as resistant to *P. zeae*. Germani (1972) reported that *Aphasmatylenchus straturatus* was associated with stunted and chlorotic pigeonpea in Burkina Faso in West Africa. *H. seinhorsti* has been found associated with poor plant growth in India.

Soybean

Soybean (*G. max* [Fabaceae, Phaseolus]), was first cultivated for food in northern China over 3000 years ago (Hymowitz, 1970). Thus, for several thousand years, people in eastern Asia have used soybean seed for animal feed and human consumption, as well as for medicinal purposes. Soybean is the leading oilseed crop produced and consumed in the world (see Table 9.2). It currently is grown in at least 70 countries, and though originally confined to temperate zones, has become important in many tropical and subtropical regions, especially in South America, the Far East and, more recently, Africa (Hymowitz *et al.*, 2015).

The majority of modern soybean cultivars have an oil content of 19–23% and a protein content of 30–40%. Most soybean meal is used as a high source of protein in animal feeds for the production of beef, fish, pork and poultry (Hymowitz *et al.*, 2015). In east Asian countries like China, Japan and Korea, soybeans are typically consumed as whole foods, usually cooked, fermented, roasted, or sprouted. In contrast, in the western hemisphere, soy is often highly processed, following cracking, dehulling, crushing, or being subjected to solvent extraction processes to separate the oils from the rest of the bean. However, attitudes are changing as vegetarian diets become more popular in places like the USA. Technology now exists that allows use of the whole bean in many foods (Hinson and Hartwig, 1977), and varieties more adapted to human consumption have been developed.

The minimum germination temperature for soybean seeds is 12°C, and rates of germination increase with warmer temperatures (Hymowitz *et al.*, 2015), whereas the optimum temperature for plant growth and development is 25–30°C. Therefore, soybeans can be produced in temperate, tropical and subtropical regions as long as soil moisture and temperature requirements are realized.

Meloidogyne

Root knot disease of soybean, caused by several *Meloidogyne* spp., has been reported from many soybean-growing areas of the world (Koenning, 2015). Three species of *Meloidogyne* (*incognita*, *javanica* and *arenaria*) account for most of the reported damage in areas with long growing seasons and warm climates. Root knot nematodes cause varying degrees of stunting, chlorosis and, in some cases, early senescence, depending on the initial population density.

The management of root knot is a necessity in much of southern USA and Brazil. *M. incognita* and *M. javanica* are widely distributed in Brazil and have caused significant yield reduction in many fields (Wrather *et al.*, 2001). Yield losses of up to 90% have been reported from Florida (Kinloch, 1974). *M. arenaria* consists of at least two races and *M. incognita* of at least four races that are identified by the ability to parasitize selected host differentials (Koenning, 2015). Populations of *M. incognita* vary in their ability to reproduce on soybean, although this may not be correlated with race. Race 2 of *M. arenaria* tends to be a more aggressive pathogen of soybean than race 1 (the groundnut race). This is important in the management of root knot in areas where soybean and groundnut are part of the crop rotation sequence.

Damage from root knot varies depending on the species involved and the population density, cultivar susceptibility and intensity of drought stress to which the host is subjected (Koenning,

2015). Because of the effect of drought stress, the greatest yield losses are likely to occur where soybeans are grown in sandy, light-textured soils. Other factors, such as soil compaction, potassium deficiency and low amounts of organic matter, also contribute to the overall loss in yield. In addition, a high incidence of root knot infection decreases the formation of nodules by *Bradyrhizobium* spp., and increases host susceptibility to vascular fungal pathogens such as species of *Fusarium*.

Symptoms

The primary symptom for identifying root knot is the presence of galls on infected roots (Fig. 9.15). The galls vary in size and number depending on the species involved and the intensity of infection. The vascular system of infected tissues is disrupted, which can inhibit water and nutrient flow through the plant. As a result, secondary symptoms develop including chlorosis and stunting, and a tendency of infected plants to wilt in the heat of the day. Patches of infected plants that often appear irregularly in the field normally spread in the direction of cultivation and surface water flow (Fig. 9.16).

Management measures

The use of crop rotation is hampered by the wide host range of all three species of *Meloidogyne* (Koenning, 2015). Soybean cultivars exist that are resistant or tolerant to some root knot species and these should be used when planting in



Fig. 9.15. Root knot galls on infected soybean roots in Alabama, USA. (Photograph courtesy of E. J. Sikora.)



Fig. 9.16. Patch of chlorosis and stunting of infected soybean plants infected with root knot nematodes. The spread of infection is in the direction of cultivation and surface water flow (Photograph courtesy of E. J. Sikora.).

nematode-infested fields. Resistance to the nematode is horizontal, and some yield loss occurs in these cultivars when populations of root knot are relatively high. Damaging levels of root knot usually occur following several years of continuous planting of the crop or other hosts in infested fields. Consequently, both preventive and corrective management practices are needed. In addition, planting and cultivating equipment must be washed free of contaminated soil, and fields should be maintained free of weeds, many of which are good hosts. Nematicides are rarely profitable for root knot management on soybean. Gramineous crops are less susceptible hosts and, when grown in rotation with soybean, reduce residual populations of *Meloidogyne* juveniles. When soil infestation levels of juveniles reach damaging levels, summer planting with a gramineous crop is beneficial, irrespective of the species of *Meloidogyne* present.

Heterodera glycines

The soybean cyst nematode (*H. glycines*) is known to occur in all major soybean production areas in the world, and ranks among the most economically destructive pathogens on this

crop (Niblack and Riggs, 2015). The nematode causes greater yield losses than any other soybean disease in the USA (Wrather and Koenning, 2009). The soybean cyst nematode likely co-evolved with leguminous and other hosts, and was well established in South-east Asia by the time agricultural soybean production began (Noel, 1992). The nematode causes severe stunting and yellowing of the foliage and, in extreme cases, plant death. Yield losses of 20–30% have been observed in the absence of symptom development (Noel, 1992). Crop losses can range from 10 to 80%, depending on rainfall, soil fertility, the presence of other diseases and nematode density (Jacobsen *et al.*, 1983).

Symptoms

Above-ground symptoms of infection by *H. glycines* may include slight to severe stunting and chlorosis, which often occurs in circular to oblate patches (Fig. 9.17), but symptoms typically are not apparent even when a 15–30% yield loss occurs (Niblack and Riggs, 2015). Although symptoms can appear suddenly, their appearance probably reflects a nematode problem that has been developing and affecting yield for several



Fig. 9.17. *Heterodera glycines* induced stunting and yellowing of the foliage. (Photograph courtesy of G. Tylka.)

cropping seasons. The lack of above-ground symptoms makes diagnosing soybean cyst nematode damage difficult. A variety of soil-borne pathogens and abiotic disorders such as nutrient deficiencies, herbicide injury or drought can produce similar symptoms. Symptoms of damage on the infected root range from slight discoloration to severe necrosis. Both nodulation by *Bradyrhizobium japonicum* and nitrogen fixation in nodules that do form are reduced in heavily infected plants. Because the symptoms are non-descript, confirmation of the presence of *H. glycines* must be based on the appearance of the adult females attached to the roots (Fig. 9.18). Young females of *H. glycines* are white or yellow, and turn brown with age. The young female is the only life stage that is easily visible without magnification. After death, the female body becomes a dark brown, lemon-shaped cyst (Fig. 9.19).

Biology

The life cycle of *H. glycines* is similar to that of other plant parasitic nematodes (see Chapter 2,



Fig. 9.18. Adult white female cysts of *Heterodera glycines* attached to the roots of soybean. (Photograph courtesy of G. Tylka.)

this volume). The optimum temperature range for development is 24–28°C, but development can occur at temperatures ranging from 18°C to 32°C (Niblack and Riggs, 2015). Females

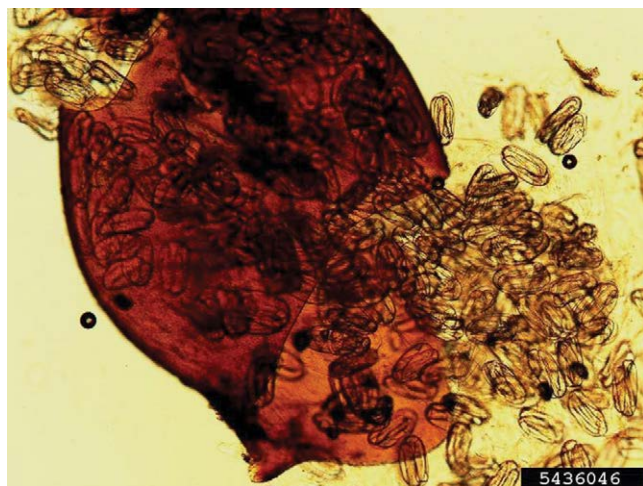


Fig. 9.19. Female *Heterodera glycines* cyst with between 100 and 600 eggs with 50–200 deposited externally in a gelatinous matrix. (Photograph courtesy of G. Tylka.)

become so large that they rupture the cortex and epidermis while remaining attached to the feeding site. Under optimum conditions, the life cycle can take as little as 22 days. The female produces between 100 and 600 eggs, with 50–200 deposited externally in a gelatinous matrix (Fig. 9.19). The remaining eggs are retained within the body and can remain viable for 12 years or more. The eggs survive due partly to protection by the cyst wall and in part to a genetically controlled dormancy. The soybean cyst nematode can be disseminated by water, wind, soil peds in uncleaned seed, and machinery (Epps, 1969b). Virtually anything that can move soil can spread this pathogen.

Disease complexes

Several wilt and root rot diseases of soybean caused by fungi are enhanced by infection with *H. glycines* (Bond and Wrather, 2004). Nematode infection has been reported to increase the incidence and severity of brown stem rot (*Cadophora gregata* syn. *Phialophora gregata*) in both resistant and susceptible soybeans (Tabor *et al.*, 2003). Moderate to high populations of *H. glycines* may be associated with sudden death syndrome of soybean (*Fusarium virguliforme*) and may increase the severity of the disease (Hartman *et al.*, 2015).

Management measures

Management of the soybean cyst nematode begins with confirming the presence and popula-

tion density of *H. glycines* in the field (Niblack *et al.*, 2002; Niblack and Riggs, 2015). Because young females may be visible on roots for only a short period of the growing season, confirmation based on soil samples is recommended. Egg counts can be used to predict the likelihood and extent of damage and yield loss from an infestation. Management should focus on improving soybean yield, reducing *H. glycines* infestation levels and preserving the yield potential of resistant cultivars.

An effective management strategy consists of rotating non-host crops with *H. glycines*-resistant cultivars with different sources of resistance. Consistent use of the same source of resistance results in the selection of a *H. glycines* population that is adapted to and able to damage that source of resistance and any cultivar developed from that source. Populations of *H. glycines* differ in their ability to parasitize resistant or tolerant soybean cultivars. These populations can be distinguished on a set of indicator lines representing the seven sources of resistance that have been used in US breeding programmes; the designations are virulence profiles known as HG types (Table 9.4). Periodic HG-type testing can aid soybean producers in long-term nematode management using resistant cultivars with different resistance levels or sources of resistance. The most modern soybean cultivars resistant to the soybean cyst nematode are high-yielding cultivars. The latter can mask the presence of an emerging nematode resistance-breaking population.

Table 9.4. Indicator lines for HG-type classification of genetically diverse populations of *Heterodera glycines*. HG types are designated by the number of the indicator line on which a nematode population can develop at a rate of 10% or more of its development on the susceptible check cultivar Lee 74.^a (From Niblack and Riggs, 2015.)

Number	Indicator line
1	PI 548402 (Peking)
2	PI 88788
3	PI 90763
4	PI 437654
5	PI 209332
6	PI 89772
7	PI 548316 (Cloud)

Note: ^aHG type 0 cannot reproduce on any source of resistance; HG type 1 is able to develop on indicator line No 1 (PI 548402); HG type 2 is able to develop on indicator line No 2 (PI 88788), and so forth. HG types designated by more than one number, such as the common HG type 2.5.7, are able to develop on indicator lines numbered 2, 5 and 7 (PI 88788, 209332 and 548316).

Unfortunately, farmers are almost completely reliant on a single source of soybean resistance (PI 88788) to soybean cyst nematode (Tylka, Iowa State University, Iowa, 2016, personal communication). Virulence to this source of resistance is increasing, and is correlated with increased reproduction on PI 88788-derived commercial varieties, leading to measurable yield losses on these resistant varieties in the field. Increased virulence of soybean cyst nematode populations on PI 88788 threatens the long-term effectiveness of soybean resistance to the nematode and the sustained profitability of soybean production in soybean cyst nematode infested fields. The genetic base of resistance in commercially available soybean varieties is currently too narrow to provide sustainable management of this pest. Long-term, sustainable management will require a multifaceted, integrated pest management approach that includes the use of non-host crops, nematode-protectant seed treatments, conventionally bred soybean varieties with resistance from multiple breeding sources, and possibly varieties with transgenic resistance to soybean cyst nematode.

Cultural practices that reduce plant stress, including weed control and providing adequate levels of fertility and moisture, should be used to

reduce damage from *H. glycines*. While soybeans are the primary host for the soybean cyst nematode, the nematode can feed on several alternative hosts, including many edible legumes such as cowpea, adzuki bean and snap beans, as well as numerous weed hosts (Riggs, 2004). Alternative hosts allow for *H. glycines* feeding and for the nematode to complete its life cycle. Late planting of soybean, or planting in wheat stubble in a double-crop situation, may reduce *H. glycines* population levels, but the reduction is usually not enough to alleviate yield losses. Nematicides applied in-furrow can reduce early season root infection, but typically do not provide season long control and may not be economical. Recently labelled nematode control products that can be applied in combination with fungicide/insecticide seed treatments are available, but they should be used with accepted *H. glycines* management practices, because of the lack of knowledge on their long-term viability or effectiveness.

Rotylenchus reniformis

R. reniformis was first identified as a pathogen of soybeans in West Africa in 1956 (McGawley and Overstreet, 2015). The nematode has a wide distribution in tropical and subtropical locations, but also survives well in temperate zones. The nematode can affect both the growth of soybeans and reduce crop yield significantly. Yield reductions in soybeans of over 30% have been attributed to reniform infestations in the southern USA. Studies have shown that soybean yields decrease significantly as the population of reniform nematodes increases (Lawrence and McLean, 1999).

Symptoms

Reniform nematodes usually produce the least distinctive symptoms of any of the nematodes that attack soybeans. Symptoms of damage caused by reniform nematode may include yellowing of the foliage, stunting of the plant, root decay, reduced pod formation and seed size, and reduced yields (Lawrence and McLean, 1999). Stunted plants are typically in areas where the soil type is poor and where plants may be stressed by other abiotic factors such as

drought or low fertility. The root systems on infected plants may be severely underdeveloped and necrotic, with fewer feeder roots. Recently infested fields may show an irregular pattern of stunted plants and an uneven growth across a field. Over a period of years, this symptom becomes less noticeable after reniform populations become more evenly distributed in the area. Reniform nematodes do not form galls on roots, which makes them more difficult to identify without magnification. However, one characteristic of roots infected with reniform is the presence of soil particles adhering to the adult females, and egg masses that are attached to the root even after they have been lightly rinsed with water (Fig. 9.20).

Disease cycle and epidemiology

Reniform nematodes are semi-endoparasitic parasites (McGawley and Overstreet, 2015). The anterior portion of females is embedded in the root, with their heads located near the vascular system; the posterior portion of the nematodes' body remains outside the root. The life cycle is relatively fast, being completed in 17–23 days at optimum soil temperatures of 27–30°C. Juvenile stages of reniform nematodes do not feed; only females have been observed to infect and feed on roots. Females take on a characteristic reniform or kidney-shaped appearance as they mature. Females deposit an average of 75 eggs in a gelatinous mass on the root surface. The eggs appear to be resistant to desiccation, allowing the nematode to survive long periods under

dry soil conditions, and possibly cold temperatures in northern growing regions.

Management measures

Some soybean varieties are moderately to highly resistant to this parasite and should be considered where reniform nematode is a problem (McGawley and Overstreet, 2015). Crop rotation is also effective in reducing reniform populations. The production of maize or grain sorghum for 1–2 years can reduce the population below economic damage threshold levels to allow for the successful production of soybeans (Lawrence and McLean, 1999). Rotation with groundnuts or rice has also been shown to reduce reniform populations effectively. A 2-year rotation with a non-host crop is sometimes necessary when reniform nematodes reach excessively high levels in a field. This is why it is important to monitor reniform populations annually at the end of a cropping season, for management of this pathogen. Where crop rotation is practised, fields should be maintained free of weeds, to prevent reproduction on potential alternative hosts. Chemical nematicides have been used successfully to decrease at-planting populations of reniform nematodes and increase yields, but the cost of this practice is prohibitive in many parts of the world.

Pratylenchus spp.

Species of *Pratylenchus* are found throughout the world and attack a wide range of crops, including soybean (Noel, 2015). Important lesion nematodes reported on soybean include *Pratylenchus agilis*, *P. alleni*, *P. brachyurus*, *P. coffeae*, *P. crenatus*, *P. hexincisus*, *P. neglectus*, *P. penetrans*, *P. pratensis*, *P. scribneri*, *P. sefaenis*, *P. teres*, *P. thornei*, *P. vulmus* and *P. zae*. *P. brachyurus* and *P. zae* are found primarily in tropical and subtropical regions. *P. brachyurus* is currently the most important nematode species in soybean production regions of Brazil, with high populations reducing yields by 30–50% (Rodrigues *et al.*, 2014).



Fig. 9.20. *Rotylenchulus reniformis* exposed anterior portion of females embedded with egg mass on the root of soybean. (Photograph courtesy of K. Lawrence.)

Symptoms

Damage from lesion nematodes can occur without noticeable symptoms in the field (Noel, 2015).

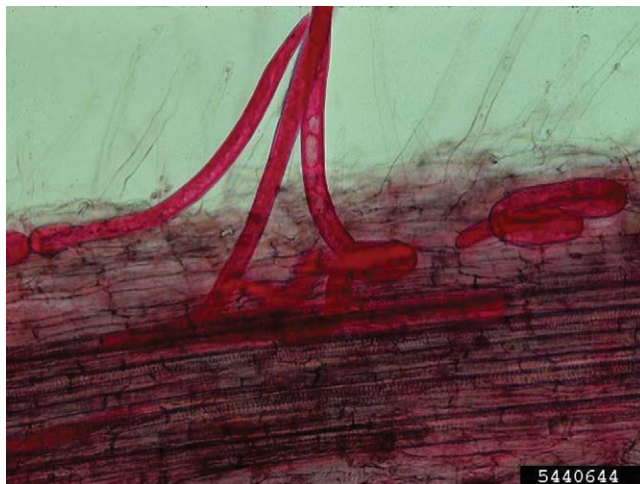


Fig. 9.21. Microscopic view of stained lesion nematode (*Pratylenchus* sp.) on the surface and feeding inside the cortical tissue of soybean roots. (Photograph courtesy of J.D. Eisenback.)

Symptoms of chlorosis and stunting from root damage can appear when accompanied by deficiencies in nutrients or soil moisture. Yield reductions are greater when high lesion nematode populations are associated with abiotic disorders such as drought or poor fertility. Lesion nematodes are migratory endoparasites that attack the root cortex (Fig. 9.21). Feeding damage is visible as dark lesions and browning of the root system, which may decrease root growth. The epidermis and cortex of severely infected roots may slough away from the stele. The degree of damage is generally related to the nematode population at the time of planting and soil temperatures.

Management measures

The control of lesion nematodes is hampered by the wide host range of its many species, and the possibility of multiple species within one field (Noel, 2015). Various monocots and dicots serve as hosts for the various *Pratylenchus* spp., thus making crop rotation ineffective for managing these nematodes in many situations. Soybean cultivars also vary in their degree of susceptibility to species of *Pratylenchus*. For instance, cultivars with Fayette in their pedigree (PI 88788 source of resistance used to manage *H. glycines*) are susceptible to *P. scribneri*, which also feeds on and reduces yield of maize. In Brazil, damage from *P. brachyurus* is reduced through crop rotation or succession planting using

non-host species (Ferraz, 2006; Inomoto, 2011). The use of non-host or nematode-antagonistic plants is seen as an important strategy since, in addition to reduced nematode populations, they help improve the physical and chemical properties of the soil (Souza and Pires, 2007). Treatment with nematicides is not an economically feasible option for management of lesion nematodes on soybean.

Black Gram, Urd, Mash

Black gram (*Vigna mungo* syn. *Phaseolus mungo*), also known as urd or mash, probably originated in India and is a bushy annual common in Asia, Africa and America. The plant, which is very similar to mung bean, is resistant to high temperatures and is reasonably drought resistant. It is often intercropped with cotton, maize or sorghum.

R. reniformis has been detected on gram in Puerto Rico (Ayala and Ramirez, 1964). In India, *H. cajani*, *R. reniformis* and *Tylenchorhynchus mashhoodi* have been found associated with the crop (Sitaramaiah, 1984) and are considered to be of economic importance. The root knot nematodes *M. incognita* and *M. javanica* are known to infect black gram in Brazil (Freire *et al.*, 1972). Root knot has also been detected on the crop in India (Nadakal, 1964). In the Rajasthan area of India, *M. incognita* was found in 54% of the 176 fields sampled (Datta *et al.*, 1987).

Economic threshold level

M. incognita and *R. reniformis* were shown to cause significant growth reductions at 1 juvenile/cm³ of soil in pot tests (Mishra and Gaur, 1981). Growth reduction increased with the level of infestation, and both nematodes reduced the number of *Rhizobium* nodules. Zaki and Bhatti (1986) reported that *H. cajani* at 1 juvenile/g of soil did not affect shoot growth, but caused reductions in root weight. Gupta and Yadav (1979) in pot studies showed that plant growth was reduced significantly by *R. reniformis* at densities of more than 2 nematodes/g of soil. Damage threshold densities for *M. incognita* have been set at 1–2 juveniles/g of soil (Mahapatra *et al.*, 1999). *M. incognita* has been shown to have a negative effect on *Rhizobium* (Chahal and Chahal, 1987; Chahal *et al.*, 1988).

Root knot nematodes also interact with soil pathogens to cause more disease than when present alone. Wilting caused by *Fusarium pallidoseum* increased greatly when in combination with *M. incognita* (Swain and Kar, 1994), and a reduction in plant growth and nodulation was detected when *M. javanica* was associated with *R. bataticola* (Fazal *et al.*, 1998).

Management measures

In a study with five crop sequences designed specifically to control *M. incognita*, black gram after mustard and rice was the most effective in reducing root knot galling (Mahanta and Phukan, 1990). The use of non-hosts and paddy rice in cropping systems will be the most effective and economical means of preventing damage. Resistant cultivars are not available, even though moderate levels of resistance have been detected in some lines (Sikora *et al.*, 2005).

Winged Bean

Winged bean (*Psophocarpus tetragonolobus*), also known as Goa bean, asparagus pea, four-angled bean, Manila bean and princess pea, originated in Asia or Africa. It is a perennial crop grown as an annual for green immature pods, seeds, tubers and leaves in the humid tropics. The crop is resistant to high temperatures and is often intercropped with sweet potato, taro,

banana, sugarcane and vegetables. It can be grown as a dry-season crop with irrigation, but is not drought resistant.

Meloidogyne

Root knot nematodes have been shown to cause serious damage to winged bean in a number of tropical countries. *M. incognita* has been reported on the crop in Papua New Guinea (Price and Linge, 1979), India (Singh *et al.*, 1979), Okinawa (Teruya *et al.*, 1984) and Nigeria (Whitehead, 1969). *M. javanica* caused damage in Papua New Guinea (Price and Linge, 1979), Brazil (Lordello and de Almeida, 1979) and Okinawa (Teruya *et al.*, 1984). Root knot nematodes are considered the most widely distributed pests of winged bean in Papua New Guinea.

A 'Meloidogyne-javanica-incognita-arenaria species complex' was responsible for severe galling to roots and tubers in the Côte d'Ivoire (Fortuner *et al.*, 1979). Species of *Meloidogyne* have also been reported from Mauritius (de Sornay, 1913) and the Philippines (Fajardo and Palo, 1933).

The distribution of the two major species attacking winged bean is influenced by temperature. *M. incognita* seems to be more predominant in the warmer coastal regions of Papua New Guinea and at lower altitudes in East Africa, whereas *M. javanica* is common in the highlands and higher altitudes (Whitehead, 1969; Price and Linge, 1979). These observations are supported by the fact that hatching of local populations occurs in a temperature range of 25–30°C for *M. incognita* and 20–30°C for *M. javanica* (Price and Linge, 1979). In the field, the juveniles penetrate the root within 1 week and females and galls develop within 4 weeks (Linge, 1976).

In the Côte d'Ivoire, the root knot nematode species complex caused heavy root galling and tuber galling so severe that they were unsuitable for consumption. An estimated 50–70% of the tubers failed to develop. Damage to the tubers was observed even at very low initial infestation levels (Fortuner *et al.*, 1979). Damage seems to be more severe on winged bean grown in the dry season (Price and Linge, 1979).

No attempts have been made to develop control measures for root knot nematodes on this crop. Resistance to *M. incognita* has not been

detected in the lines screened to date (Duncan *et al.*, 1979; Singh *et al.*, 1979; Valdez, 1981; Phukan and Hazarika, 1985). Breeding lines with resistance to *M. javanica* have been identified (Valdez, 1981).

Other nematodes of winged bean

A number of plant parasitic nematodes of unknown importance have been found associated with winged bean (Teruya *et al.*, 1984; Bridge, 1987).

Nematode Parasites of Other Food Legumes

A large number of food legumes have not been discussed in detail in this chapter. Most of these crops were considered to be of local importance and only a few reports of nematodes associated with the crop were found. The plant parasitic nematodes that have been found associated with these food legumes have been compiled from major lists (see Table 9.3), and this list is not to be considered complete. Species of root knot nematodes, cyst nematodes (*Heterodera*) and lesion nematodes parasitize many of these crops. The stem and bulb nematode, *D. dipsaci*, the reniform nematode and *Belonolaimus* probably cause damage on many of these food legumes and are of local importance on the crops listed. The species that have been reported to attack a number of these crops and that may be economically important are *H. glycines*, *M. arenaria*, *M. incognita*, *M. javanica* and *R. reniformis*.

Conclusions and Future Prospects

Food legumes are grown worldwide as a commercial crop for the marketplace as well as on small family farms, where they are often part of the family food basket. We reviewed the nematode pests and plant health solutions facing both types of growers. Commercial farms have access to: quality seed, fertilizer, irrigation, mechanization and pesticides, as well as links to extension support or online information to solve their nematode problems. The production of food legumes in

the tropics and subtropics, however, is dominated by small, resource-limited family farms of <2 ha. The majority of these low-income farmers have limited access to: seed, fertilizer, pesticides, or extension support. This form of subsistence agriculture is common in Saharan Africa, India, South America and South-east Asia. Yield losses due to nematodes can have a significant impact on the availability of food for such family farms. Far too little is known about the presence, distribution and losses caused by nematodes in subsistence agriculture (Noe and Sikora, 1990).

Nematode management is often not practised consciously, or is limited by poorly organized cropping systems often set up without regard for nematode control. In most cases, the resource-limited growers are unaware that they have a problem – the so-called unseen enemy remains unseen. Nematode-resistant varieties would be an important solution to their problems, but often they are not available for many food legumes. Nematodes are often neglected in selection programmes, as breeders screen their germplasm for other targets. Although lines with resistance to nematodes have been identified repeatedly in many food legume crops, these traits have not been transferred to cultivars suitable for field use. The exceptions to the rule are soybean and chickpea, where resistance has been obtained for *H. glycines* and different species of *Meloidogyne*.

The benefits of crop rotation for nematode management in temperate regions, where typically one crop per year is grown, are easy to demonstrate and transfer to growers, especially those that currently practise a near monoculture system. Conversely, on commercial and small farms in the tropics and subtropics, rotation patterns consist of two or more crops grown sequentially in a 12-month period. Designing nematode management strategies based on rotations under these conditions is a challenge to nematologists, especially in cases where there is a lack of resistant cultivars. Bridge (1987) suggested a number of approaches to nematode control in different cropping systems. Sikora *et al.* (2005) have outlined a wide range of tools for integrated nematode management (see Chapter 23, this volume). In areas where randomly mixed cropping occurs, nematode pressure is probably never reduced below the damage threshold level,

due to the presence of multiple host crops distributed evenly throughout the farm (Noe and Sikora 1990). In such cases, only resistant cultivars can have a positive effect on nematode damage.

Many attempts have been made to use organic amendments to manage nematode densities in soil, with different degrees of effectiveness. Most of these practices, when used alone, have not been shown to be of practical importance in food legumes, often for economic reasons (Sikora and Greco, 1990; Sikora *et al.*, 2005). However, it has been shown that organic amendments stimulate soil biodiversity and thereby increase the level of suppressiveness to nematode pests (see Chapter 3 and 23, in this volume). Understanding and being able to measure the antagonistic potential in soils and then taking advantage of this suppressiveness in management programmes needs more study (Sikora, 1992; Sikora *et al.*, 2005).

A wide spectrum of biological control agents has been tested for nematode control, including: arbuscular mycorrhizae, rhizobacteria, fungal egg pathogens and microbial endophytes (Sikora and Greco, 1990; Sikora *et al.*, 2005; Stirling, 2014). Biologically based management approaches, however, have not yet been adopted by commercial growers, or often only on a small local scale, and are therefore only selectively included in this revision. However, biological seed treatments with antagonistic bacteria are being used on soybean in the USA. This form of biological enhancement of seed could have a major impact on food legume production.

Nematicides are still too costly for use on the vast majority of food legumes. However, the development of a new generation of nematicides that are less toxic and are effective will reach the marketplace in the near future. The combination of biological and chemical seed treatment or IPM on the seed can lead to a reduction in nematode early root penetration and increased yield (see Chapter 23, this volume).

There are a number of publications that list resistant cultivars and lines of food legumes, and these are listed in the chapter on food legumes in the previous edition of this book (Sikora *et al.*, 2005). Although many breeding lines have shown different degrees of resistance to important nematodes, only a handful of resistant cultivars are currently available to farmers

(Sikora *et al.*, 2005). In many instances, screening for resistance or tolerance to food legumes has not been developed adequately. There is an imperative need to expand the screening process to include nematode pests so that resistant and/or tolerant cultivars are developed for all food legumes.

Diagnosis

Root knot nematodes

Species of root knot nematodes can usually be recognized by the presence of root galls, which, with most species affecting food legumes in tropical and subtropical climates, are large. To the untrained eye, root knot galls often resemble *Rhizobium* nodules. The latter, however, are distinct knots of root tissue attached to the surface of the root, which can be detached easily from the root surface, whereas galls are swellings arising on all sides of the root. Above-ground symptoms vary from stunting to chlorosis. Plants may wilt when exposed to moisture stress and in cases involving interrelationships with fungal wilt diseases. In some plants, early senescence has been reported. A pictorial guide to nematodes of agricultural importance has been published by Bridge and Starr (2007).

Cyst-forming nematodes

The presence of white, lemon-shaped or round females, 0.4–0.8 mm in length, attached to the root surface is the most characteristic symptom of this group of nematodes. Knowledge of the day degrees, the sum of temperature above the minimum temperature needed for activity that coincides with the appearance of adult females on the root surface, can be used to simplify detection in field survey work. The presence of white females on the root surface is a simultaneous verification of parasitism.

The presence of cysts in soil samples is an indication that a cyst nematode problem is present in the cropping system; it does not indicate which crop or weed is being parasitized. Cyst colour varies greatly, from white to dark brown. Colour can be species specific, but usually indicates cyst age, with dark brown an indication of an old cyst.

The extraction of cysts from a predetermined quantity of soil and determination of the total number of eggs and juveniles found in the extracted cysts is the most exact measure used to determine nematode densities and to study population dynamics.

Stem and bulb nematode

The date selected for soil sampling and the depth of sampling are important in determining nematode densities when looking at nematodes in the upper soil layers only.

On broad bean, leaf spot symptoms caused by fungal diseases can be confused with necrosis induced by the stem nematode. The spots on infested seed cannot be used as a diagnostic characteristic, because they can be caused by insect damage and water spotting.

For routine studies and experimentation, Hooper (1983a) suggested soaking 150 g of seed in 500 ml of water overnight. To prevent introduction of the nematode into nematode-free areas, a high level of nematode extraction accuracy from seed is necessary. Augustin and Sikora

(1989b) suggested first soaking and then maceration of the seed and extraction on a modified Baermann tray (see Chapter 4, this volume).

Lesion nematodes

Species of *Pratylenchus* cause distinct small brown to black lesions on the root surface of many food legumes. They can often be seen with a simple magnifying lens in the field, or with a field microscope. In extreme cases, the lesions coalesce to form large necrotic lesions. The nematodes can be extracted as outlined in Chapter 4, this volume.

Reniform nematode

Reniform nematodes are semi-endoparasitic parasites (McGawley and Overstreet, 2015). The anterior portion of females is embedded in the root, with their heads located near the vascular system; the posterior portion of the nematodes' body remains outside the root. Females take on a characteristic reniform or kidney-shaped appearance as they mature. Females deposit an average of 75 eggs in a gelatinous mass on the root surface.

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10 Nematode Parasites of Vegetables*

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Vegetables are an important dietary component rich in unique nutritional elements such as protein, vitamins, minerals and fibre. Previously, such highly perishable commodities were available on a seasonal basis only or in restricted growing regions. With mass transportation and modern processing schemes, however, these vegetables are now readily available, both nationally and internationally.

Asia, Africa, South America and Central America are the major commercial producers of vegetables in the tropics. However, the vast proportion of subsistence farmers across the tropics and subtropics will produce vegetables seasonally for home consumption, in addition to peri-urban and urban vegetable producers, who constitute an important source of fresh, perishable vegetable produce for supply to every urban centre. Many other important crops can also be used as vegetables; for example, sweet potato, or taro and the leaves of cassava, which are discussed in Chapters 7 and 8, this volume, respectively. Similarly, many of the crops covered under food legumes (see Chapter 9, this volume), such as cowpea, garden pea, mung bean, broad bean and haricot bean, which are also often considered to be vegetables, will not be dealt with here. In [Table 10.1](#), the overall level of production of a number of vegetable crops in these four tropical regions is presented.

In most areas of the world, vegetable consumption and production have expanded rapidly in the past three decades, with production significantly outpacing population growth in the four regions listed since 1990, when the first version of this book was published ([Table 10.2](#)). There have also been significant shifts in the amounts of specific vegetables produced in a region.

Surprisingly, the total amount of ‘fresh vegetables’ produced for the market as a percentage of total production has, with the exception of Central America, actually decreased since 1990 ([Table 10.3](#)). Vegetables are nowadays processed more intensively to create higher-value convenience food, but also to allow long-distance transport and better storage conditions in tropical and subtropical climates.

Increased production is associated with major advances in production and processing technology. In addition, modern breeding methods, supported by new molecular techniques, have substantially shortened the development time for breeding cultivars with plant resistance to nematodes, insects, diseases and abiotic stresses, as well as improving the nutritive value of vegetable crops.

Across tropical and subtropical regions, the control of nematodes is, essentially, a prerequisite for successful vegetable production.

*A revision of the chapter by R.A. Sikora and E. Fernández in the second edition.

Table 10.1. Area in 1000 ha, yield in metric tonnes and total production in 1000 metric tonnes for selected vegetables in regions with largely tropical and subtropical climates in 2015. (From FAOSTAT database at: www.fao.org/faostat.)

Vegetable	Asia			Central America			South America			Africa		
	Area	Yield	Production	Area	Yield	Production	Area	Yield	Production	Area	Yield	Production
Aubergines	1739	27	46,615	2	66	136	2	10	23	83	21	1773
Cabbages	1733	30	52,740	22	16	360	14	29	371	222	18	4117
Carrots and turnips	711	32	23,167	17	27	474	54	21	1125	102	19	1961
Cauliflower and broccoli	946	19	18,419	37	15	552	98	16	157	18	23	427
Chillies and peppers	1222	17	21,376	137	17	2374	38	13	503	368	8	2887
Cucumbers and gherkins	157	40	62,756	18	39	711	10	16	167	254	5	1258
Garlic	1217	18	22,181	8	10	77	41	9	366	57	11	641
Lettuce and chicory	827	20	16,530	21	21	441	22	15	333	17	19	320
Onions	130	21	2705	8	11	81	20	9	181	46	14	348
Spinach	847	26	22,113	2	11	20	2	18	42	5	21	110
Tomatoes	2821	35	99,205	105	37	3873	131	51	6731	976	20	19,177

Table 10.2. Comparison of regional population and changes in total area, yield and production for all vegetables and melons between 1990 and 2015/2013 in regions with largely tropical and subtropical climates and percentage increase. (From FAOSTAT database at: www.fao.org/faostat.)

	Population (number)			Area (1000 ha)			Yield (t/ha)			Production (1000 t)		
	1990	2015	%	1990	2013	%	1990	2013	%	1990	2013	%
Asia	3,100,917	4,393,296	42	19,293	42,847	122	15	20.5	37	299,003	876,262	193
Central America	111,449	172,740	55	571	849	49	13	19.4	49	7676	16,489	145
South America	296,170	418,447	41	1143	1531	34	13	16.8	29	14,291	25,716	80
Africa	622,440	1,186,178	91	3745	7421	98	9	10.8	20	33,130	80,060	142

Table 10.3. Comparison of fresh vegetable production as a percentage of total vegetable production between 1990 and 2013 in 1000 metric tonnes. (From FAOSTAT database at: www.fao.org/faostat.)

	1990			2013		
	Total	Fresh	Percentage	Total	Fresh	Percentage
Asia	299,003	116,787	39	876,262	242,637	27
Central America	7676	421	6	16,489	1148	7
South America	14,291	2934	21	25,716	4197	16
Africa	33,130	9570	29	80,060	18,693	23

For example, vegetable crops produced for the fresh market have traditionally relied heavily on soil fumigation as a standard practice for nematode control. This was reflected by the fact that 75% of the soil fumigant methyl bromide, now withdrawn from the market, was used for soil treatment, with over 40% for vegetable production (Anon., 1998).

In large-scale peri-urban and intensive commercial production operations, crop protection often uses cutting edge technology and is highly developed, while in developing countries the numerous small, resource-limited vegetable growers often lack coordinated plant protection support, leading to insufficient crop pest and disease management.

Cultivation Techniques

Depending on the demographic structure and economic development of a region, vegetable production in the subtropics and tropics varies from the gathering of fruit, leaves and tubers found among the natural vegetation to technically advanced, large-scale commercial field and protected production.

The increase in the importance of vegetables is especially evident in countries with rapidly expanding populations, where, since 1990, urban and peri-urban vegetable production has expanded by 142 and 193% in Africa and Asia, respectively (Table 10.2). In addition, the protected cultivation of vegetables using plastic mulches, tunnels or plastic greenhouses has expanded significantly in many countries in the tropics and subtropics, for both domestic and export markets. The greatest expanse of protected vegetable production lies in the Mediterranean region of Europe, with 100,000 ha of greenhouses and 299,879 ha under plastic tunnels and mulches, with Spain cultivating 46,000 ha and Italy 61,775 ha under greenhouse production. Japan, China and Turkey, as well as many countries in North Africa, also have significant areas under protected cultivation (Hanan, 1998). It is important to note that 5000 ha of the greenhouse production is in soilless culture (Cantliffe and Vansickle, 2001).

Nematodes of Vegetables

Plant parasitic nematodes are an extremely important limiting factor in vegetable production. The role plant parasitic nematodes play in limiting vegetable production, however, depends to a large extent on the farming system employed. In general, nematodes will be less important under the more extensive and varied growing systems typical of shifting cultivation and multiple inter-crop farming systems in subsistence agriculture, as well as in widely spaced rotations of some commercial farming systems. However, nematodes are very important in more intensive production systems; for example, in protected cultivation where monocropping is practised, or in field production systems where soil fumigation is followed by sequential cropping of a series of susceptible hosts (Taylor, 1976). Crops grown in Senegal under local cropping conditions were not affected by root knot, while neighbouring irrigated vegetable fields were heavily infested (Netscher, 1978). However, pressure on land and available resources has in many countries shifted production from small, multiple-crop production units toward more intensive production systems, even at the small farm scale. Peri-urban production of vegetables for local city markets has increased to enormous levels in the past 30 years. Similarly, the export of high-value vegetables from tropical

and subtropical production zones to satisfy the highly lucrative spring, autumn and winter markets in temperate zones around the world has had a major impact on vegetable production, creating major nematode problems. This has led to a high dependence on the use of soil fumigant and synthetic nematicides in many regions.

From an ecological standpoint, crops grown in shifting cultivation and in the other multiple intercropping systems common to rural subtropical and tropical areas still have much in common with the natural flora. The distribution of important plant parasitic nematodes associated with the natural vegetation is clustered. The distributions of the species that survive the drastic shift to multiple intercropping are also heterogeneous, even if polyphagous species are present. Extensive damage by nematodes, therefore, is extremely rare in the crops produced directly after clearing. Exceptions include instances where nematode-infected planting material, in the forms of seedlings or tubers, is used for planting (Bridge, 1987).

Nematode problems are facilitated and perpetuated through limited availability and access to nematode- and disease-free seedlings. In many cases, these seedlings are produced under suboptimal conditions, and are often infected with nematodes, insects and diseases (Singh *et al.*, 2000). Since commercial nurseries producing high-quality vegetable seedlings often do not exist in Africa and Asia, local farmers using traditional methods produce their own planting material from nurseries planted in the open in a small, untreated area of their field. The rule, and not the exception, results in poor-quality seedlings, infected with pests and diseases.

Multiple intercropping systems, although initially reflecting the natural flora, will promote nematode population build-up over time. The population increase will depend on the type of nematodes initially present and on the percentage of susceptible plants per unit area (Noe and Sikora, 1990). Surveys in Niger and Benin showed that seedlings in smallholder nursery beds were regularly infected with root knot (R.A. Sikora, Bonn, 2004, unpublished data). Consequently, plants were infected from an early age, while the infected transplants additionally transferred the infection to the field. Within a very short timespan, the entire farm was threatened.

Damage intensity usually increases slowly with time in the multiple intercropping system, as

compared with the rapid increase in damage encountered in large-scale vegetable production where monoculture or near monoculture is practised. Large differences also exist between the plant parasitic nematode communities of tropical and temperate regions, where vegetable crops have been recorded as a host for at least one of the most frequently occurring species of root knot nematodes, *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne hapla* and *Meloidogyne arenaria*. Important temperate parasites such as *Ditylenchus dipsaci* and species of *Heterodera* tend only to be of local importance in the tropics, but can be problematic in the cooler seasons in the subtropics and on vegetables grown at higher altitudes. Conversely, root knot nematodes that predominate in tropical regions are uncommon in temperate regions (Taylor and Sasser, 1978). Greater crop damage is to be expected in warmer regions and in summer crops than in cooler growing regions or in the upland tropics (Noe and Sikora, 1990).

Root knot nematodes, which can increase quickly to damaging levels within a few seasons on susceptible crops, are so common in subtropical and tropical vegetable production that they are frequently taken to represent 'nematodes' in general. Other economically important nematode species, in particular cyst nematodes but also *Rotylenchulus reniformis* and *Paratrichodorus minor*, are regularly overlooked because of indistinct symptoms, and are often neglected by plant protection agencies. Nematodes such as *Heterodera schachtii*, *Nacobbus aberrans*, *Belonolaimus longicaudatus*, *Xiphinema* spp. and *Tylenchorhynchus brassicae* have, however, been shown to be serious pests. Feldmesser *et al.* (1971) estimated that the loss in yield caused by all plant parasitic nematodes on 24 vegetable crops in the USA was approximately 11%.

Meloidogyne

Root knot nematodes (*Meloidogyne* spp.) are universally associated with vegetable production across the globe. Although nearly 100 nominal species of *Meloidogyne* have been described to date (Wesemael *et al.*, 2013), four species are of particular economic importance, *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. In addition, *Meloidogyne enterolobii* (syn. *M. mayaguensis*),

a highly pathogenic and aggressive invasive species, is emerging as an economically important species worldwide (Villar-Luna *et al.*, 2016), due in part to its pathogenicity on hosts resistant to *M. incognita* and *M. javanica* (Castagnone-Sereno, 2012). Of 1000 *Meloidogyne* populations collected in 75 countries, 53% were identified as *M. incognita*, 30% as *M. javanica*, 8% as *M. arenaria*, 8% as *M. hapla* and 2% as *Meloidogyne exigua* or other species (Taylor and Sasser, 1978), although *M. enterolobii* is increasingly being detected (Pagan *et al.*, 2015; Janssen *et al.*, 2016).

M. incognita, *M. javanica*, *M. arenaria* and *M. hapla* have the widest host ranges. *M. incognita* and *M. javanica* are commonly found in the tropics, whereas *M. arenaria* is more common in the subtropics. *M. hapla*, a species commonly known from temperate regions, is increasingly found in the cooler upland but also in the warmer lowlands of the tropics, such as the Central Rift Valley of Ethiopia (Meressa *et al.*, 2014).

M. enterolobii, a species first described from Hainan Island in China, has been detected from a growing list of countries, including Benin, Brazil, Burkina Faso, China, Costa Rica, Côte d'Ivoire, Cuba, Florida (USA), Kenya, Malawi, Mexico, Mozambique, Nigeria, Senegal, South Africa, Thailand, Vietnam and Venezuela (Fig. 10.1). *M. enterolobii* reacts similarly to *M. incognita* race 4 in standard differential host tests (Brito *et al.*, 2004) but overcomes the *Mi-1*, *N* and *Tabasco* resistance genes of tomato and pepper genotypes, which thus limits their use for

controlling root knot nematodes (Brito *et al.*, 2007). This species is increasingly reported from crop hosts, including over 30 vegetable crops in tropical and subtropical agriculture, such as bell pepper, tomato, cabbage, broccoli, aubergine, celery, parsley, watermelon, sweet potato and pumpkin, but also beet, potato, tobacco, guava, papaya, maize, coffee and yam. It is now regarded as an A2 quarantine pest in European and Mediterranean countries (Rodríguez *et al.*, 2003; www.eppo.int/QUARANTINE/quarantine.htm (accessed 12 January 2018)). Some resistant cultivars of carrot, lettuce and brassica plants have been reported in Brazil (Rosa *et al.*, 2015).

Meloidogyne floridensis, a species described from Florida, reproduces on root knot resistant peach rootstocks, and has been shown to parasitize tomato, watermelon and cotton, but not tobacco, pepper, groundnut, verbena, aubergine, squash or basil (Kokalis-Burelle and Nyczepir, 2004). This species resembles *M. incognita*, *Meloidogyne christiei*, *Meloidogyne graminicola* and *Meloidogyne hispanica* but LM and SEM observations show a clear morphological difference from these species (Handoo *et al.*, 2004). Isolates varied in their reproductive fitness on maize, pepper or tomato, but none of the isolates tested multiplied on cotton, groundnut or soybean (Stanley *et al.*, 2009). The importance of this species appears geographically limited. The main species of *Meloidogyne* found parasitizing vegetables are listed by crop in Table 10.4.



Fig. 10.1. Tomato roots heavily galled by *Meloidogyne enterolobii*. (Photograph courtesy of S. Kiewnick.)

Table 10.4. Root knot nematodes, *Meloidogyne* species, associated with major vegetable crops in the subtropics and tropics.

		<i>arenaria</i>	<i>incognita</i>	<i>javanica</i>	<i>chitwoodi</i>	<i>hapla</i>	<i>mayaguensis</i>	<i>floridensis</i>
<i>Allium ascalonicum</i>	Shallot		•	•				
<i>Allium cepa</i>	Onion	•	•	•	•			
<i>Allium porrum</i>	Leek		•	•				
<i>Allium sativum</i>	Garlic		•					
<i>Allium schoenoprasum</i>	Chives			•				
<i>Amaranthus hybridus</i>	Spinach (bajem)		•	•				
<i>Amaranthus viridis</i>	African spinach			•				
<i>Apium graveolens</i>	Celery		•	•			•	
<i>Basella alba</i>	Spinach		•	•			•	
<i>Beta vulgaris</i>	Beetroot	•	•		•	•	•	
<i>Brassica chinensis</i>	Chinese cabbage		•					
<i>Brassica nigra</i>	Black mustard	•						
<i>Brassica oleracea</i> var. <i>viridis</i>	Kale	•		•				•
<i>Brassica oleracea</i> var. <i>botrytis</i>	Cauliflower	•		•			•	
<i>Brassica oleracea</i> var. <i>capitata</i>	Cabbage	•	•	•		•	•	
<i>Capsicum annuum</i>	Sweet pepper, chilli	•	•				•	•
<i>Capsicum frutescens</i>	Cayenne pepper	•	•	•				
<i>Celosia argentea</i>	African spinach	•	•	•		•		
<i>Citrullis vulgaris</i>	Watermelon	•	•	•			•	
<i>Cucumis meta</i>	Melon	•	•	•				
<i>Cucumis sativus</i>	Cucumber	•	•	•				•
<i>Cucurbita maxima</i>	Squash	•	•	•				•
<i>Cucurbita pepo</i>	Pumpkin	•	•	•			•	
<i>Daucus carota</i>	Carrot	•	•	•	•	•		
<i>Ipomea aquatic</i>	'Spinach' (kangkung)					•		
<i>Lactuca sativa</i>	Lettuce	•	•	•		•	•	
<i>Lagenaria siceraria</i>	Bottle gourd	•		•				
<i>Lagenaria vulgaris</i>	Calabash	•	•	•				
<i>Luffa cylindrica</i>	Sponge gourd		•	•				
<i>Solanum lycopersicum</i>	Tomato	•	•	•	•		•	•
<i>Momordica charantia</i>	Balsam pear	•	•				•	
<i>Petroselinum crispum</i>	Parsley	•	•			•	•	•
<i>Sechium edule</i>	Chayotte			•				
<i>Solanum melongena</i>	Aubergine	•	•	•			•	•
<i>Solanum tuberosum</i>	Potato	•	•	•	•	•	•	
<i>Solanum nigrum</i>	Black nightshade	•	•	•				

Meloidogyne ethiopica causes serious economic damage to several vegetable crops, mainly cabbage, bell pepper, lettuce, cucumber and tomato. Highly susceptible potato cultivars have been reported from Brazil (Medina *et al.*, 2014). The differential host test demonstrated that *M. ethiopica* could reproduce on tomato, tobacco, pepper and watermelon, but not on cotton or groundnut (Regina *et al.*, 2004), and therefore reacted similarly to *M. incognita* race 2. Unlike the aforementioned four key *Meloidogyne* species, information on the extent of its damage to these crops is not available. However, the fact that this nematode could result in a minimum yield of 6% fresh top weight and 0% root weight compared to non infested controls of grape seedlings (Di Vito *et al.*, 2009) suggests that it could be even more pathogenic to annual vegetable crops. This tropical species is reported from several countries in Africa and South America, as well as Turkey, Slovenia and Greece (Medina *et al.*, 2014).

Symptoms of damage

Above-ground symptoms due to *Meloidogyne* infestation are not distinct from other factors that cause root malfunction, but may often occur clustered. Galling of the root system is the primary symptom associated with *Meloidogyne* infection. The severity of root galling depends on the nematode species, population density and plant species or cultivar. The size and form of the gall depends on the species involved, the number of nematodes in the tissue, the host and plant age. In cucurbits, galls can be extremely massive and fleshy, whereas in most other vegetables, galls tend to be small to large and firm (Figs 10.2 and 10.3). In general, larger galls are formed in the warmer lowland tropics compared with the more temperate upland tropics. Symptoms of root knot on monocotyledonous crops, such as onion and leek, are usually discrete, with egg masses protruding from the root surface. Some species do not cause galling at all, such as *Meloidogyne artiellia*, with females and egg masses exposed on the root surface, similar to cyst nematodes.

The root systems of plants that are severely infected with *Meloidogyne* are reduced to a few heavily galled roots, with secondary roots almost extinct. Such roots are seriously hampered in their ability to access and uptake water and nutrients. Plants wilt rapidly, especially under arid/dry conditions, and are often stunted

and/or chlorotic. Seedling infection can result in high plant mortality rates. Additionally, severe losses in quality can be caused by infection of the taproot (Fig. 10.4). Root and tuber crops such as carrot, beets and radish can sustain immense losses due to the poor marketability of forked and deformed tubers (Fig. 10.5). Tuber infection also severely limits storage potential, in that these taproots tend to desiccate more quickly and are prone to rotting from fungal infection associated with nematode gall degradation.



Fig. 10.2. Massive galling by *Meloidogyne incognita* on tomato roots in Morocco. (Photograph courtesy of R.A. Sikora.)



Fig. 10.3. Galls produced by *Meloidogyne incognita* on pepper roots in Cuba. (Photograph courtesy of E. Fernández.)



Fig. 10.4. Carrots heavily infected by *Meloidogyne incognita* in Nigeria. (Photograph courtesy of D. Coyne.)

Biology and life cycle

Second-stage juveniles of *Meloidogyne* enter the roots behind the root tip and then migrate intercellularly along the vascular cylinder to a feeding site. Secretions by the nematode transform the initial parenchymatic root cells into a multinucleate and hypertrophied feeding cell, the so-called giant cell. The increase in cell numbers (hyperplasia) and cell size (hypertrophia) results in the formation of a root gall. As a consequence of the disorganized root tissue, nutrient uptake is disturbed. With continuous feeding from the giant cells, the juveniles develop through three additional moults to the adult stage (Fig. 10.6). Females usually burst with their posterior protruding from the root tissue to lay their eggs in a gelatinous egg mass, each containing 300–500 eggs, which can be seen with the naked eye as light brown dots on the surface of the galled tissue.

The life cycle of *Meloidogyne* species varies depending on species, temperature and host plant. Temperature optima for species occurring



Fig. 10.5. Biforking and deformation of celery by *Meloidogyne incognita* in smallholder horticulture, Nigeria. (Photograph courtesy of D. Coyne.)



Fig. 10.6. Different juvenile stages of *Meloidogyne incognita* in tomato cv. Moneymaker following staining with acid fuchsin. (Photograph courtesy of T. Thoden.)

in the warmer tropics (e.g. *M. incognita*, *M. javanica*) are 25–30°C compared with 18–25°C for species in cooler regions (e.g. *M. hapla*). The first group usually does not survive freezing temperatures, whereas *M. hapla* can easily withstand periods of ground frost. Under favourable conditions, *M. incognita* can develop eight or more generations per year, considering that one cycle is completed in

19 days at 30.6°C and 43 days at 21.8°C (Cuadra, 1983). Ploeg and Maris (1999) estimated for the duration of one life cycle for *M. incognita* a base temperature of 10.1°C and a required heat sum of 400°C. In comparison, the number of generations for *M. hapla* usually ranges between 4 and 6, assuming a base temperature of 8°C and a heat sum of 450°C. *Meloidogyne* species can also adapt to temperature conditions. For example, temperature optima for populations of *M. javanica* from Australia and California were 25–30°C and 32–34°C, respectively (Ferris and Van Gundy, 1979). The ability of *Meloidogyne* to adapt to local conditions probably also explains its successful dissemination. For example, *M. hapla*, commonly known to occur in temperate regions, is increasingly reported from Africa, Central America and South America (<http://www.cabi.org/isc/datasheet/33244> (accessed 28 November 2017); Meressa *et al.*, 2014). *M. hapla* was considered to occur in areas having a temperature between –15°C during the coldest month and 27°C during the warmest month (Taylor *et al.*, 1982), but now occurs in regions with temperatures exceeding 40°C.

Environmental factors affecting parasitism

Soil texture and structure are directly related to water-holding capacity and aeration, and influence nematode survival, emergence and disease severity. Sikora (1989), studying paddy rice–vegetable cropping systems, detected severe root knot damage on vegetables grown in sandy soils after paddy, but a total absence in heavy clay soils. Soil type also influences nematode distribution and movement (Taylor *et al.*, 1982). For example, in sandy soils, juveniles move horizontally and vertically up to 75 cm in 9 days (Prot, 1977), whereas movement decreases with increasing clay content of the soil, with no movement in soils at more than 30% clay content (Prot and Van Gundy, 1981). Soil pH is another factor influencing *Meloidogyne* species. Most species survive and reproduce at pH levels ranging from 4.0 to 8.0 (Ferris and Van Gundy, 1979). Lower pH levels can suppress the population of *M. incognita*. A fact that, according to Chiu *et al.* (2012), explained the reduction of *M. incognita* on water spinach following the application of chemical fertilizers by reducing the soil pH. Wallace (1966) reported that the emergence of

M. javanica was greatest between pH 6.4 and 7.0 and inhibited below pH 5.2. However, a pH of 4.5 is rather common in many tropical soils, but does not seem to prevent *Meloidogyne* build-up.

Biological races

Within *Meloidogyne* species, great physiological variability exists. Riggs and Winstead (1959) demonstrated that when populations of *M. incognita* and *M. arenaria* were inoculated on to resistant cultivars of tomato, enough selection pressure was exerted by the cultivar that within a short time resistant-breaking populations called ‘B races’ were created. Sasser (1966) found that when different populations of the same species were inoculated to certain hosts, they often reacted differently. Currently, the use of host differentials (Hartman and Sasser, 1985; Robertson *et al.*, 2009) allows determination of the races for the four main *Meloidogyne* species (Table 10.5). Based on the results obtained with several hundred *Meloidogyne* populations, Sasser (1979) concluded that there was considerable uniformity in host response and that resistance-breaking races were not common. In studies in Cuba with over 200 populations from a wide spectrum of hosts, *M. incognita* races 1, 2 and 3, *M. arenaria* race 2, as well as *M. javanica* and *M. hapla* were detected (Pérez *et al.*, 2001). However, Robertson *et al.* (2009) reported 13 *Meloidogyne* populations collected from horticultural regions of Spain that did not fit into the published race scheme and thus identified new races as *M. incognita* races 5 and 6, *M. javanica* races 1 and 5 and *M. arenaria* race 3. The development of resistance-breaking pathotypes/populations continues as new cultivars carrying new resistance sources enter the market and exert selection pressures. Nonetheless, resistance is a key management tool on which farmers depend, and therefore it is essential to understand and determine the pathotype occurring in the field.

Survival and means of dissemination

Root knot nematodes are obligate parasites. Consequently, the absence of suitable host plants for prolonged periods ultimately leads to their disappearance; juveniles rapidly deplete their energy reserves and eventually die, with up to

Table 10.5. Differential host test identification of the most common *Meloidogyne* species and races.
(From Hartman and Sasser, 1985; Robertson *et al.*, 2009.)

	Tobacco	Cotton	Pepper	Watermelon	Groundnut	Tomato
<i>Meloidogyne incognita</i>						
Race 1	–	–	+	+	–	+
Race 2	+	–	+	+	–	+
Race 3	–	+	+	+	–	+
Race 4	+	+	+	+	–	+
Race 5 ^a	–	–	–	x	x	+
Race 6 ^a	+	–	–	x	–	+
<i>Meloidogyne arenaria</i>						
Race 1	+	–	+	+	+	+
Race 2	+	–	–	+	–	+
Race 3 ^a	+	–	+	x	–	+
<i>Meloidogyne javanica</i>	+	+	–	+	–	+
Race 1 ^a	+	–	–	x	–	+
Race 5 ^a	–	–	–	x	–	+
<i>Meloidogyne hapla</i>	+	–	+	–	+	+

Notes: Cotton, cv. Deltapine; tobacco, cv. N.C.95; pepper, cv. Early California Wonder; watermelon, cv. Charleston Gray; groundnut, cv. Florunner; tomato, cv. Rutgers. ^aCotton cv. DP6; pepper, cv. Sonar; tomato, cv. Malmalade.

– = a resistant host; + = a susceptible host; x = not tested.

90% population decline within 2–3 months. However, a proportion of *Meloidogyne* eggs can remain in diapause, ensuring perpetuation of the species (de Guiran, 1979). Various weeds may, however, act as alternative hosts, and so care is required if cultivating non-hosts as a management practice. In general, conditions favourable for plant growth are equally favourable for *Meloidogyne* reproduction.

The survival of *Meloidogyne* is influenced mostly by soil moisture content and, to a lesser extent, by temperature. A prolonged dry season with high soil temperature will ultimately prove lethal to juveniles and eggs. In field soil, the number of juveniles decreased from an initial infestation of approximately 1000 nematodes/100 ml of soil to zero after 12 weeks, when the soil gradually dried out (de Guiran, 1979). Similar effects were observed in the dry season in Senegal, where no *Meloidogyne* was detected in the top 20 cm at the end of the dry season and only 0.9% of the initial population at 20–40 cm, where some moisture remained (Demeure, 1977).

Dissemination of *Meloidogyne* mainly takes place when juveniles or eggs are transported from infested to uninfested areas. Wind-borne dissemination of root knot nematodes has been reported (Orr and Newton, 1971), and is probably a major factor during the monsoon season in

Asia and on the Indian subcontinent. Dispersal through irrigation water has been reported from the USA (Faulkner and Bolander, 1970) and Spain (Tobar and Palacios, 1974), especially when the irrigation water is taken from nearby creeks, canals or water holes, as *Meloidogyne* juveniles are easily washed with runoff water into those reservoirs. Further spread occurs with soil adhering to footwear and agricultural implements. Plant residues harbouring *Meloidogyne* should not be applied to the field, not even after composting; a few nematodes will always survive (Fernández *et al.*, 1994). Dispersal over great distances and international borders occurs by the movement of infected plants. Therefore, quarantine measures should be strictly enforced to avoid the spread of *Meloidogyne* to new regions.

Disease complexes

Disease complexes formed by the interaction of *Meloidogyne* with other pathogens are common in the tropics and subtropics and have been reviewed intensively by several authors (Pitcher, 1963; Powell, 1971a,b; Taylor, 1979; Webster, 1985; Manzanilla-López and Starr, 2009). Most of the work covers the interaction with soil-borne fungal and bacterial pathogens. For example, tomato plants wilt more quickly in the presence of

Fusarium oxysporum (Fig. 10.7). Resistance in tomato cultivars to *F. oxysporum* f. sp. *lycopersici* was reduced in the presence of *Meloidogyne* (Sidhu and Webster, 1977). The disease complex caused by *M. incognita* and *Rhizoctonia solani* on okra and tomato is well established and causes severe yield loss (Saikia and Bhagawati, 2014). In many cases, the fungi are only weak pathogens that require simultaneous infestation by *Meloidogyne* to develop their full damage potential. Van Gundy *et al.* (1977) even demonstrated that leachings of nematode-infected plants applied to tomato inoculated with *Rhizoctonia* resulted in the appearance of severe rot, indicating the intricacy of microbial interactions with nematodes in the rhizosphere and their importance for root health.

Similar to fungal pathogens, soil-borne bacterial pathogens also cause disease complexes with *Meloidogyne*. Best documented is probably the interaction between *Meloidogyne* and *Ralstonia solanacearum* causing bacterial wilt on tomato (Chindo *et al.*, 1991). Within this respect,

Deberdt *et al.* (1999) indicated that at least one gene governing part of the bacterial wilt resistance was closely linked to the *Mi* gene in tomatoes for root knot resistance. An interaction between *Meloidogyne* and bacterial canker caused by *Corynebacterium michiganense* has been reported by de Moura *et al.* (1975). Nesha and Siddiqui (2013) demonstrated that inoculation of *M. javanica* 15 days prior to *Pectobacterium carotovorum* or *Xanthomonas campestris* predisposed carrots to a higher reduction in plant dry weight than by either pathogen alone. In general, suppression of such disease complexes by the control of *Meloidogyne* will result in significant yield increases.

Economic importance

Meloidogyne is the most important plant parasitic nematode on vegetables. Infected plants are characterized by reduced development, stunting, overall delayed maturity, early die-back, low yields and low quality of marketable produce.



Fig. 10.7. Synergistic yield losses caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici* on tomato, Kenya. (Photograph courtesy of J. Mwangi.)

Meloidogyne damage to root systems also has a multifaceted effect on restricting access to water and nutrients. Severity of damage can be species specific, and can also vary by crop and environmental conditions. Consequently, general estimates for yield losses are quite difficult to make. However, estimates have been provided for defined conditions. For example, Ravichandra (2014) estimated yield losses caused by *M. incognita* to be 29% on tomato and 15% on pepper, and 2% for *M. hapla* on okra, *Abelmoschus esculentus*. Yield losses can range between 30 and 60% on aubergine and 50% on cantaloupe and watermelon. For intensive commercial production with up to four susceptible crops per year, *Meloidogyne* infestation can lead to total crop failure. However, eliciting the specific role of *Meloidogyne* in crop loss becomes complicated where crops suffer from simultaneous and multiple attack by other pests and diseases, or even abiotic constraints, a situation common in subtropical and tropical conditions. Nematode damage is especially severe in protected cultivation, where susceptible crops are repeatedly planted in the same bed.

In the USA, yield on plots infested with *M. incognita*, treated with a fumigant nematicide and planted with beans, summer squash, okra or cucumber, increased 128, 180, 507 and 1175%, respectively (Johnson, 1985). These figures clearly demonstrate the economic impact of these nematodes on vegetable production under intensive production systems. Root knot nematodes, however, also cause severe crop loss in multiple cropping systems in subsistence smallholder vegetable farms where they have been considered as the most important biotic constraint (Sikora and Fernández, 2005; De Waele and Elsen, 2007). Crop loss assessment under these conditions is generally lacking though, which is complicated due to the reasons outlined. Their attack undermines resistance to other pests and diseases, predisposes hosts to other pathogens and results in the inappropriate use of pesticides (James *et al.*, 2010).

Economic threshold level

In general, all factors affecting nematode reproduction will, in turn, also affect the extent of damage on a certain host plant. Therefore, economic threshold levels vary depending on nematode

species, host plant and environmental conditions (Ferris *et al.*, 1986). A comprehensive overview on tolerance limits and minimal yield for several vegetable crops under different environmental conditions is given by Greco and Di Vito (2009). It is clearly shown that the tolerance limit for *Meloidogyne* is generally less than 1 egg or juvenile/cm³ soil and the minimum yield tends to be zero. A few crops show tolerance, such as parsley or capsicum pepper, with a minimum yield of 0.5 and 0.16 for *M. incognita*, respectively (Aguirre *et al.*, 2003), although this is also cultivar dependent. In addition, Di Vito and Zacheo (1991) and Di Vito *et al.* (1991) reported a tolerance limit of 0.55 eggs or juveniles/cm³ soil for both susceptible and resistant tomato cultivars, whereas for artichoke seedlings the tolerance limit was 1.1, and for cabbage 0.5. Watermelon showed a minimum yield and tolerance limit of 0.63 and 0.20 eggs or juveniles/cm³ soil for *M. javanica*, respectively (López-Gómez *et al.*, 2014). Tolerance limits for *M. incognita* on courgette (*Cucurbita pepo* subsp. *pepo* convar. *giromontina*) were 0.324 and 0.006 eggs or juveniles/cm³ soil in spring and autumn cropping cycles, respectively (Vela *et al.*, 2014). In the San Joaquin Valley of California, USA, the number of juveniles in samples taken from sandy loam soils has been used for estimating potential yield loss in processing tomato production areas (Table 10.6). These figures are given here to be used as guidelines for estimating possible loss in other growing areas. In general, these densities should be considered as a starting point for those working with threshold levels, which then need to be adapted to the local conditions.

Root knot nematode management

The variation in vegetable production systems, ranging from large-scale commercial production through intensive smallholder peri-urban conditions to subsistence farming, prevents the development and recommendation of a single management strategy that is applicable to all situations. For example, the subsistence farmer frequently utilizes a mixture of local crops and landraces to ensure a minimum yield, but often does not follow modern management recommendations. These farmers often will not grow an unfamiliar nematode-resistant cultivar or do not have access to such planting material. On the other hand, a commercial plantation manager will not hesitate to

Table 10.6. Effect of root knot nematode populations on processing tomato yield in San Joaquin Valley, California, USA, in a sandy loam soil. (From Anon., 1985.)

Number of root knot juveniles/kg soil		
Autumn samples	Spring samples	Percentage of normal yield
≤160	≤25	100
310	50	98
620	100	95
940	150	91
1250	200	88
1560	250	85
1870	300	82
2190	350	79
2500	400	77
2810	450	74
3120	500	72
3440	550	69
3750	600	67
4060	650	65
4370	700	63
4690	750	61
5000	800	60
5310	850	58
5620	900	56
5920	950	55
6250	1000	53

utilize resistant cultivars, recommended nematicides or reliable biological control options to protect a valuable crop (Noling, 2003; Ornat and Sorribas, 2008). In the first case, crop management improvement is more difficult to implement; in the latter case, it is available to most growers having access to current technology.

A number of strategic reviews have been published that focus on specific regions or on nematode management in vegetable production (Johnson and Fassuliotis, 1984; Netscher and Sikora, 1990; Johnson, 1998; Sikora, 2002; Ornat and Sorribas, 2008; Coyne *et al.*, 2009). It should be noted that many of the techniques used for *Meloidogyne* management in vegetables are used to control other plant parasitic nematodes affecting a wide array of crops (Nickel, 1984; Brown and Kerry, 1987; Barker *et al.*, 1998; Whitehead, 1998). This is especially important where multiple species of economically important nematodes affect the vegetable crop. To be effective, however, it is absolutely necessary to

combine as many components as possible into a management system.

In the commercial production of high-value fresh vegetables, reliance on fumigant and synthetic nematicides remains the preferred method of management, especially where multiple vegetable crops are grown sequentially per year. However, with the withdrawal or restricted use of most nematicides and fumigants, alternative nematode management strategies are required (Sikora, 2002; Sikora *et al.*, 2004), with increasing interest in biologically based options to reduce reliance on synthetic nematicides (Hallmann *et al.*, 2009).

Once large populations of *Meloidogyne* have developed in a field, it is difficult to reduce the populations below threshold levels without the use of effective management tools used in a strict and logical order. For example, although *M. javanica* densities were reduced to low levels – following either two non-hosts or a resistant/poor host – and aubergine yield increased significantly, nematode population density still rose to high levels by the end of the first season (Netscher, 1981). In this respect, root knot nematode control remains a perpetual ‘never-ending battle’, requiring serious attention to develop and maintain sustainable production systems, especially under more intensified conditions.

Physical methods of nematode management

QUARANTINE: control strategies should be preventive rather than curative in nature and aimed from the onset at preventing the build-up of high population densities. Quarantine, if practised correctly, can add greatly to prevent the introduction of a pest into a new country or region. The spread of economically important nematodes, such as *Belonolaimus*, *Nacobbus* and *Radopholus*, as well as important species of root knot have been excluded or strongly limited in the past.

Important to vegetable production is the increasing threat of highly damaging species of root knot: *M. ethiopica*, *Meloidogyne chitwoodi*, *M. enterolobii* and *M. floridensis* (Handoo *et al.*, 2004; Elling, 2013). In order to protect local production, effective quarantine laws and border inspections are needed for all four species.

At the national level, monitoring systems can be used to prevent the local spread of nematodes by close scrutiny of commercial vegetable

nurseries and nurseries on large production farms. For example, in Cuba, soil and organic amendments used in vegetable nurseries are sampled and stringently assessed for plant parasitic nematodes (E. Fernández, unpublished).

PLANTING DATE: the fact that the minimum temperature required for *M. incognita* development in the root is significantly lower than the minimum 'activity threshold' of 18°C for *M. incognita* second-stage juveniles (Roberts *et al.*, 1981) has been used to identify suitable planting dates for root knot control. Changing the normal date of planting to coincide with low soil temperature was considered an important control tactic on carrots (Roberts, 1987), and could be used to limit nematode damage on vegetables in cool upland tropical regions.

FALLOW: obligate parasites such as *Meloidogyne* require a host plant to survive. Therefore, bare fallow is an effective measure for managing root knot (Johnson and Fassuliotis, 1984; Brown and Kerry, 1987; Netscher and Sikora, 1990). Especially in areas where the climate is characterized by a prolonged and severe hot dry season, fallow during the dry season, with soil tillage to dry the soil and destroy weeds, followed by non-hosts during the wet season, will result in significant reductions in *Meloidogyne* populations (Johnson and Fassuliotis, 1984). Under some conditions, fallowing has provided equal or better control than rotation with non-host or cover crops (Kinloch and Dunavin, 1993). Of course, bare fallow has to be economical and acceptable to the grower. The negative effects of bare fallow on soil health and soil conservation may limit its use in some situations.

ROOT DESTRUCTION: as *Meloidogyne* can survive on roots left in the soil after harvest, galled roots should be eliminated by uprooting and destruction. The spread of the nematode to the following crop will be suppressed and the overall population density reduced. It has been estimated that, when soil temperatures are high, for each month that the root system survives, a ten-fold increase in root knot nematode densities can result (Coyne *et al.*, 2009).

SOIL TILLAGE: generally, major effects of soil tillage in managing *Meloidogyne* are not expected.

Johnson *et al.* (1983) reported that standard tillage practices did not have significant effects on nematode densities in intensive vegetable cropping systems. However, Eo *et al.* (2007) reported a greater speed of *M. incognita* juvenile migration in tilled soil (bulk density 0.64 g/cm³) than in non-tilled soil (0.86 g/cm³), concluding that conventional tillage might enhance the migration and reinfection of crops by *M. incognita*. A different and more promising aspect of tillage is seen in heaping soil up around the stem base of established seedlings with topsoil. Covering the stem base of tomato and pepper plants with topsoil 30 days after transplanting led to the production of adventitious roots on the buried stems, which were less affected by *Meloidogyne* and improved plant vigour (E. Fernández, unpublished).

FLOODING: *Meloidogyne* juveniles are killed after exposure to anaerobic conditions that begin in the soil a few days after flooding (Padgham *et al.*, 2003); the effect on root knot eggs is unknown though. Therefore, juvenile densities drop significantly when soils are flooded for prolonged periods, such as in systems where paddy rice is a normal component of the rotation (Fig. 10.8). Thames and Stauer (1953) demonstrated that flooding of rice fields for 3 months provided acceptable control of root knot nematode for two succeeding vegetable crops. Similarly, *Meloidogyne* densities were lower on susceptible dry-season crops in paddy rice rotations than in upland areas in the Philippines (Castillo *et al.*, 1976). The level of galling decreased significantly with increasing clay content of the soil, indicating that soil type played an active role in population reduction under flooded conditions.

In Florida, flooding alternated with drying during the summer has been recommended for vegetables grown on muck soils to reduce root knot nematode densities. According to Noling (2003), alternating 2- to 3-week cycles of flooding with drying seems to be more effective than long, continuous flooding cycles. Combining flooding and solarization can even enhance nematode control, as shown for *M. arenaria* on tomato in Florida (Sotomayor *et al.*, 1999). However, it should be noted that concerns about water conservation would limit the use of flooding in some countries (Noling and Becker, 1994). In addition, the availability of water and



Fig. 10.8. Vegetable production in rotation with paddy rice in Taiwan. (Photograph courtesy of R.A. Sikora.)

the ability to control water levels are also a limiting factor in many areas where vegetables are grown.

ORGANIC AMENDMENTS: it is well known that the incorporation of large amounts of organic material into the soil reduces root knot densities. Compost, oilseed cakes, coffee husks, neem, marigold leaves, crustacean skeletons, sawdust, urea, chicken manure, bagasse, among others, have been used successfully (Singh and Sitaramaiah, 1966, 1967; Sikora *et al.*, 1973; Muller and Gooch, 1982; Sikora, 1992; Stirling, 2014). Control may be due to: (i) toxic compounds present in the organic material, as in neem; (ii) non-toxic compounds such as residual sugar in bagasse; (iii) toxic metabolites produced during microbial degradation; or (iv) enhancement of nematode antagonists.

Chitin amendments have received much interest in that they stimulate the antagonistic potential in soil toward nematodes and induce plant resistance (Main *et al.*, 1982; Rodríguez-Kábana *et al.*, 1987; Spiegel *et al.*, 1987). Similarly, neem-based oil cakes and related products applied alone (Singh and Sitaramaiah, 1966, 1967) or in combination with biocontrol agents,

such as *Pochonia chlamydosporia* (Rao *et al.*, 1998a,b) and *Trichoderma harzianum* (Reddy *et al.*, 1998), provided significant levels of *Meloidogyne* control.

However, there is relatively little information on the true economics and practical impact of this technology at the grower level. Although the use of organic amendments for effective nematode control is often limited by availability, and in some cases by the large quantities needed, they will reduce nematode population densities to varying degrees, depending on circumstances.

SOLARIZATION AND SOIL HEATING: the lethal temperature for control of plant parasitic nematodes is considered to be around 45°C. Heating the soil either with dry or steam heat has been used extensively in protected cultivation for root knot nematode management, but the high costs involved can limit its use drastically.

Mulching/tarping the soil surface with clear plastic sheeting will trap solar energy and increase soil temperatures by 5–10°C above ambient temperature. In warmer regions, this can be sufficient to reach lethal temperatures for plant parasitic nematodes (Katan, 1981; Whitehead,

1998). The technique is most effective in regions where high levels of solar energy are available for extended periods and when the soil is moistened, which helps to conduct the heat. However, the limited depth to which lethal heat penetrates often restricts control to the upper 5–10 cm layer. Although this may be sufficient to avoid damage in the first year, the effect may not be long lasting.

Solarization could be effective in contained raised-bed production, as used in many peri-urban production systems. Solarization applied in the summer in Morocco before the next tomato crop in plastic greenhouses led to a 99% reduction in *M. javanica* densities when compared with the controls (Eddaoudi and Ammati, 1995). Similar results were obtained in India following solarization for 6 weeks in the summer months, with reductions in *M. incognita* and *Pythium aphanidermatum* (Reddy *et al.*, 2001). When a resistant cultivar followed solarization, tomato production was double that of the susceptible cultivar in solarized soil. Solarization for 40–60 days during the Mediterranean summer increased yield equal to that of methyl bromide treatment (Noto, 1994).

Solarization reduces root knot, *Verticillium* wilt and weeds in autumn crops in Florida, even though the climatic conditions are not considered ideal for soil solarization (Overman and Jones, 1986). Similar results were obtained in Cuba in peri-urban agriculture and in small-holder production units using solarization under suboptimal conditions between July and September (Fernández and Labrada, 1995). The use of solarization under suboptimal conditions, however, needs to be ascertained economically for each situation.

Soil solarization combined with dazomet or calcium cyanamide controlled root knot and increased tomato yield (Fiume and Parisi, 1995). Similarly, solarization combined with carbofuran increased tomato yields by 96% and solarization with neem cake by 52%, coupled with a significant reduction in *M. javanica* (Sharma *et al.*, 1996). Solarization for 2–4 weeks, combined with cadusafos or fenamiphos, was considered a sustainable alternative to methyl bromide fumigation in greenhouse tomato in Cyprus (Ioannou *et al.*, 2002).

Although solarization can control root knot nematode damage effectively, it also affects soil biota populations and their activities negatively

as a consequence of repeated treatments (Scopa *et al.*, 2009), which in the long term may prove counterproductive.

BIOFUMIGATION: in the strict sense, biofumigation refers to the release in soil of biocidal compounds, principally isothiocyanates, when glucosinolates in cruciferous crop residues are hydrolysed following mulching (Kirkegaard *et al.*, 1998). The loss of traditional soil fumigants generated interest in breeding brassicas such as canola or fodder crops high in glucosinolates for simultaneous use in pest and disease control. The efficacy of biofumigation in suppressing plant parasitic nematodes in field trials has been variable (Ploeg, 2008). Results range from high levels of suppression (e.g. Rahman and Somers, 2005) to no suppression at all (e.g. Stirling and Stirling, 2003). Vervoort *et al.* (2014) found no differences between the biofumigation crop and wheat, and therefore concluded that the observed changes in nematode assemblages were related to intense mechanical disturbance, the incorporation of green manure and the absence of host plants, rather than to the release of isothiocyanates. Many cruciferous plants, however, are also good hosts of some important species of *Meloidogyne*, and therefore cannot be used. The highest amount of glucosinolates is reached at the flowering stage, when *Meloidogyne* has already completed its life cycle.

In general, most plant material, when mulched into soil, will provide some level of nematode control. Volatile substances produced through microbial degradation of organic material will result in toxic activity toward a soil-borne pest or disease (Bello, 1998). Biofumigation under these circumstances is greatest when there is an optimum combination of organic matter, high soil temperatures and adequate moisture to promote microbial activity. In tropical and subtropical production systems, plastic mulch and drip irrigation can improve the effectiveness of biofumigation. For example, fresh marigold as an amendment is used effectively in root knot management in protected cultivation in Morocco. Mature plants are incorporated into beds during the summer, the beds fitted out with drip irrigation and then covered with plastic mulch for biofumigation (R.A. Sikora and H. Kaak, unpublished) (Fig. 10.9).



Fig. 10.9. In Morocco, *Tagetes patula* is grown as green manure, chopped, incorporated into the soil and covered with black tarp to control *Meloidogyne incognita* before tomatoes are planted. (Photograph courtesy of R.A. Sikora.)

A further development of biofumigation is anaerobic soil disinfestation. This method is based on supplying labile carbon (C), for example as warm-season cover crops, to stimulate microbial-driven anaerobic soil conditions in moist soils covered with polyethylene mulch (Butler *et al.*, 2012).

Control due to any form of biofumigation is probably the result of multifaceted mechanisms including: (i) non-host or trap cropping, depending on the host status of the plant used; (ii) lethal temperature due to microbial activity; (iii) nematicidal action of toxic by-products produced during the degradation of organic matter; (iv) stimulation of antagonists in the soil after biofumigation.

Cropping-based nematode management systems

Crop management is designed to attain high yields while simultaneously reducing nematode,

insect, disease and weed problems, reduce erosion and improve soil fertility. In the tropics and subtropics, vegetable production systems are extremely diverse, with production over a 12-month growing season varying in structure from: (i) sequential cropping of 2–5 susceptible vegetable crops in one field without a break crop; (ii) rotation of one or more vegetable crops with a non-host; (iii) production of one vegetable crop and one cover crop or a weed fallow; or (iv) multiple cropping with vegetables intercropped with non-host crops.

Each production system has different requirements when it comes to combatting root knot nematode infestations. In addition, the rotation crops used by a grower are planted for different reasons, which can vary greatly between the tropics and the subtropics. Selection is often dependent on the main cash crop in the cropping system.

Nematode control achieved with crop management is attained by mechanisms including:

starvation, trap cropping, antagonism, stimulation of soil antagonistic potential and/or different degrees of biofumigation. Conversely, in commercial production, where fumigation is often heavily relied on, sequential cropping of susceptible vegetable crops is practised, and rotation may not even be considered.

HEALTHY TRANSPLANTS: all crop nematode management strategies are rendered useless if transplants are infected with root knot, since early root infection leads to severe crop loss. Seedling substrates in nurseries must be free of root knot nematodes in order to reduce dissemination into root knot-free production areas. Ideally, seedlings should be raised in trays using sterile/inert 'clean' substrates. However, where farmers prepare their own seedbeds (Fig. 10.10), they should select sites with minimum infection potential, such as sites not previously planted to host plants or previously flooded during the wet season; previous paddy fields, for example (Bridge, 1987; Sikora, 1988).

If no substrates free of root knot nematode exist, available substrates need be treated accordingly. Chemical disinfestation is a common and effective practice in large production operations, whereas other methods must be considered for subsistence farming. Soil can be heated in drums or on old sheets of metal over open fires before being used for seedling production. Solarization of small quantities of soil sandwiched between pieces of plastic can also be effective. Heating soil in direct sunlight and drying reduces root knot densities drastically (Fernández *et al.*, 1994).

Pouring boiling water on to beds can also be effective in eliminating root knot nematodes from tomato seedbeds and is used by farmers in Bolivia. The water is heated on wood fires immediately alongside the seedbeds. This method is recommended by Centro de Investigación Agrícola Tropical (CIAT), Santa Cruz, Bolivia (P. Franco, CIAT, 2003, personal communication). Moistening seedbeds and then setting alight debris on the surface can also create sufficient heat to kill nematodes (Coyne *et al.*, 2009).

The production of seedlings in soilless systems may be an option for larger units. Special care needs to be taken to avoid any introduction of root knot into the closed water circulation system, which can spread rapidly in the greenhouses.

Control may require thorough cleaning of all containers and pipes with the need for new nematode-free seedlings. Nematode control in the irrigation water has been attained with heat and UV radiation (Runia, 1995; Hallmann *et al.*, 2004).

NON-HOST CROPS: non-host crops are defined here as crops that do not allow nematode reproduction. However, it should be noted that the roots of some non-host crops can react to root knot penetration with local necrosis and, in the case of very high initial nematode densities, the roots can be badly damaged and crop loss encountered. None the less, rotation with non-host crops of any type is the most important measure used for root knot management worldwide (Nusbaum and Ferris, 1973; Netscher and Sikora, 1990; Barker, 1991; Rodríguez-Kábana, 1992; Johnson, 1998). Numerous rotations have been proposed to reduce the impact of root knot nematodes in vegetable cropping systems (Page, 1979; Johnson and Fassuliotis, 1984; Sikora *et al.*, 1988).

In the tropics, especially in Asia, some effective rotations are predominantly composed of cruciferous crops, followed by a smaller number of highly susceptible crops. Rotations designed in this manner can be used effectively to reduce root knot nematode densities, even in high-intensity sequential plantings. Vegetables considered moderately susceptible or tolerant to root knot were cabbage, cauliflower and onion in Mauretania (Netscher and Luc, 1974), all cruciferous crops, onion and leek in Malawi (Bridge and Page, 1977) and broccoli, cauliflower, cabbage and onion in Bangladesh (Page, 1979). Amaranthus and chilli were considered resistant in Bangladesh (Page, 1979), and onion and amaranthus were moderately resistant in Niger (Sikora *et al.*, 1988). Fernández *et al.* (1998) separated crops into four groups: very susceptible: tomato, aubergine, lettuce, melon, cucumber, squash, okra; moderately susceptible: cabbage, cauliflower; slightly susceptible: onion, garlic; and non-hosts: mint, sesame, sorghum (Fernández *et al.*, 1998). The classification of crops by this general reaction to infection seems to be independent of the *Meloidogyne* species concerned, but can vary from one population of a species to another (Netscher, 1970).

Taking advantage of these differences, Kanwar and Bhatti (1993, 1994) recommended



Fig. 10.10. Seedbeds used for producing transplants should be free of plant parasitic nematodes. (Photograph courtesy of D. Coyne.)

the rotation: tomato, onion, resistant tomato and okra to control *M. javanica*. Alternatively, they suggested a rotation based of tomato, garlic and ridge gourd (*Luffa acutangola*), or a rotation of

cauliflower, garlic and brown sarsan (*Brassica campestris* ssp. *oleifera*), the latter effective in reducing nematode densities. A rotation of sesame, maize, groundnut, sorghum, cabbage,

velvet bean and then resistant sweet potato was effective in controlling *M. incognita* in Cuba (Fernández *et al.*, 1992, 1998). Root knot densities on tomato following sesame were reduced up to 75% compared with rotation with sweet potato.

Root knot nematodes, however, are extremely polyphagous; therefore, relatively few non-host plants are available for control through crop rotation. Unfortunately, there are many reports of *Meloidogyne* populations parasitizing plants that have been reported as non-hosts. Groundnut, for example, is often considered a non-host of *M. incognita* and *M. javanica* (Netscher, 1975), but is attacked by *M. javanica* in Zimbabwe (Martin, 1956) and the USA (Minton *et al.*, 1969). Recommended use in one locality, therefore, often needs retesting in another.

Plants considered good hosts of a *Meloidogyne* species in one part of the world may not necessarily be hosts from another part of the world (Southards and Priest, 1973). Lamberti (1979a) tested 12 populations of *M. incognita* in southern Italy, with the level of galling differing up to fourfold on tomato depending on the origin of the population. Consequently, all crops being considered for rotation must be tested for host status to local populations before rotation schemes are recommended for the field.

Further care must be taken with regards to the composition of root knot species present in a field. *Meloidogyne* populations are often composed of multiple species that may require different approaches for control.

TRAP CROPS: in trap cropping, a good host crop with good root growth capacity is planted for a short duration of time to ensure good nematode penetration, and then the roots are removed from the soil or destroyed by physical or chemical (herbicides) means, killing the developing sedentary juveniles. Trap cropping, which was developed originally by Julius Kühn (1881) to control cyst nematodes in sugarbeet, has been recommended by Potter and Olthof (1993) for the management of nematodes in vegetable crops.

Short-cycle, susceptible crops are often used in traditional vegetable rotations, where they control root knot, often without the farmer's knowledge of the concept of trap cropping. In West Africa, black nightshade *Solanum nigrum*, a leafy vegetable, is continually grown

sequentially in the same raised bed on a 3- to 4-week cycle. As toxic properties develop with age, only young plants are consumed. The crop must be harvested with the root system attached, enabling the consumer to age the plant. Root removal from the soil after 3 weeks ensures trapping and root knot death before eggs are laid (R.A. Sikora, unpublished data).

In Cuba, lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*) are used as a root knot trap crop in organic peri-urban production. The lettuce is harvested with the root system intact 30–32 days after planting and the roots are discarded before marketing, resulting in the trapping and death of large numbers of root knot juveniles (Cuadra *et al.*, 2000).

COVER CROPS: cover crops are considered here to be non-hosts of the particular root knot species targeted in the field that are used mainly to protect the soil from erosion, improve soil organic matter levels and water-holding capacity, suppress weeds and provide some nematode control. They may also be used for animal fodder or grazing, or as a green manure crop. As non-hosts, cover crops will reduce root knot densities. However, they also stimulate microbial activity after incorporation into the soil, facilitating antagonists and the microbial formation of nematocidal compounds in the soil. They are also an excellent source for anaerobic soil disinfestation (see the section on biofumigation).

A number of non-host crops, such as velvet bean (*Mucuna pruriens*), horse bean (*Canavalia ensiformis*) and joint vetch (*Aeschynomene americana*), have been used successfully for *Meloidogyne* control in southern USA (McSorley *et al.*, 1994 a,b). *Mucuna deeringiana* ploughed into the soil 3 months after planting gave effective control of *M. incognita* races 1 and 4 on tomato, beans or maize, and strong yield increases in tomato (Acosta *et al.*, 1995). Similarly, *M. pruriens* controlled *M. incognita* when planted 3 months prior to a short-term vegetable crop such as lettuce (Quénéhervé *et al.*, 1998). Resistant fodder radish cultivars effectively controlled *M. chitwoodi* (Rehiyani and Hafez, 1998; Teklu *et al.*, 2014). The enormous potential of *Brassica* species as cover crops for *Meloidogyne* control is reviewed extensively by Fourie *et al.* (2016). Some fodder and green manure crops that could be used as cover crops in rotations are listed in Table 10.7.

Table 10.7. Reaction of some fodder crops and green manures often considered non-hosts to *Meloidogyne* species.

Plant	<i>Meloidogyne arenaria</i>	<i>Meloidogyne javanica</i>	<i>Meloidogyne incognita</i>
Aeschynome	–	–	+
<i>Arachis hypogaea</i>	+ ^a	+	+
<i>Crotalaria fulva</i>	–	+	+
<i>Crotalaria grahamiana</i>	–	+	+
<i>Crotalaria retusa</i>	–	+	+
<i>Crotalaria usaramoensis</i>	–	–	+
<i>Eragrostis curvula</i>	–	+	+
<i>Glycine javanica</i>	–	–	+
<i>Indigofera hirsuta</i>	–	–	+
<i>Panicum maximum</i>	–	+	+
<i>Stylosanthes gracilis</i>	–	+	+

Notes: ^aSusceptible to many populations; + = resistant; – = not tested.

The use of elephant grass, *Pennisetum purpureum*, as mulch or the cultivation of *Brachiaria plantaginea* led to significant reductions in galling and damage over continuous tomato, especially in the *P. purpureum* treatment (Matsumoto *et al.*, 2002). This plant produces large amounts of biomass, and as a mulch greatly stimulates microbial activity in the soil.

ANTAGONISTIC CROPS: plants antagonistic to nematodes produce nematocidal compounds (Grainge and Ahmed, 1988; Jairajpuri *et al.*, 1990; Pandey *et al.*, 2003) and constitutively release toxic substances during their growth or upon nematode infection to actively kill the nematode. In the strict sense, antagonistic plants will reduce nematode populations to a greater extent than a non-host plant.

The best studied plant is *Tagetes* that releases thiophenes (Marotti *et al.*, 2010), which are assumed to be toxic to root knot (Uhlenbrock and Bijloo, 1959; Varma *et al.*, 1978; Zavaleta-Mejia *et al.*, 1993), although this still needs to be proven. Nevertheless, *Tagetes* is an excellent crop for root knot control, whether in an antagonistic capacity or through its non-host status. Sellami and Cheifa (1997) reported that *Tagetes erecta*, grown 2.5 months prior to tomato, reduced root knot densities in greenhouses. Castro *et al.* (1990) demonstrated that crop rotation and soil incorporation of *T. erecta* significantly reduced *M. incognita* root galling and increased yield. Ploeg (1999) demonstrated that *Tagetes patula*, *T. erecta*, *Tagetes signata* and a *Tagetes* hybrid reduced galling in a subsequent susceptible tomato crop compared

with the tomato–tomato rotation. In field tests, *T. patula* cv. Single Gold and *Tagetes* hybrid cv. Polynemao increased tomato yield 50% over a fallow treatment (Ploeg, 2002).

Furthermore, castor bean, marigold, mustard, sesame and sunn hemp, used in rotations, led to reduced *M. incognita* densities on tomato, with marigold having the greatest impact (Swamy *et al.*, 1995). Sesame and castor bean have also shown promise in southern USA (McSorley *et al.*, 1994b). Sunn hemp, a common cover crop and green manure, is also considered antagonistic to root knot (Kumar *et al.*, 2012). *Crotalaria longirostrata*, when grown as a cover crop and incorporated into the soil, reduced *M. incognita* and *M. arenaria* galling of tomato (Villar and Zavaleta, 1990), with incorporation more effective than simultaneous interplanting of the two crops. Similarly, sunn hemp and *T. patula*, strip-tilled for 3 months, significantly controlled *M. incognita* in bitter melon (*Momordica charantia*) (Marahatta *et al.*, 2010). Conversely, concomitantly planting *T. erecta* with tomato, green bean or cowpea in the greenhouse had only a slight effect on galling and no effect on *M. incognita* infection (El Hamawi and Mohamed, 1990). These results indicated that toxic by-products of microbial degradation were involved in control but not toxic exudates from the plant itself. *M. incognita* reproduction was equally reduced on *Crotalaria spectabilis* compared with tomato plants with the *Mi* gene (Esparrago *et al.*, 1999). A comprehensive list of antagonistic crops suppressive to *Meloidogyne* is provided by Coyne *et al.* (2009).

RESISTANCE: the use of resistant cultivars should provide the foundation of nematode management as one of the most economical and environmentally safe methods for controlling root knot nematodes. Comprehensive reviews of most aspects of resistance to *Meloidogyne* should be consulted for more detailed information (Fassuliotis, 1979; Cook and Evans, 1987; Roberts, 1992; Johnson, 1998; Williamson, 1998; Hussey and Janssen, 2002; Williamson and Roberts, 2009; Seid *et al.*, 2015; Barbary *et al.*, 2016). However, just a few sources of single gene resistance against *Meloidogyne* in vegetables are known. Resistance genes have been described for carrot (*Mj-1*), pepper (*Me1*, *Me3*, *Me4*, *Me7*, *Mech1*, *Mech2*, *N*) and tomato (*Mi-1*–*Mi-9*), all targeting the tropical species *M. incognita*, *M. javanica* and *M. arenaria* (Williamson and Roberts, 2009; Barbary *et al.*, 2016). Many, however, occur in wild species and are not yet available in cultivars. For example, of the nine resistance genes detected in wild tomato accessions, so far *Mi-1* only is available in cultivated tomatoes. This *Mi-1* gene, one of the best-characterized resistance genes, was originally incorporated into tomato via embryo rescue from a cross between the resistant wild species *Solanum peruvianum* and the cultivated tomato *Solanum lycopersicum* Sorribas *et al.* (2005) showed that growing an *Mi-1* resistant tomato cultivar in plastic houses naturally infested with *M. incognita* increased profits by €30,000/ha compared with a susceptible cultivar, and by €8800/ha over the susceptible cultivar when soil was fumigated with methyl bromide. Although *Mi-1* is highly effective and used extensively worldwide against *M. incognita*, *M. javanica* and *M. arenaria*, it is ineffective at soil temperatures >28°C and against *M. hapla* and *M. enterolobii*, which are both important on tomato. Nowadays, virulent populations occur in many regions worldwide, limiting the use of resistant cultivars (Kaloshian *et al.*, 1996; Eddoudi *et al.*, 1997; Williamson and Roberts, 2009; Tzortzakakis *et al.*, 2016).

Different resistance genes behave differently. For example, of the three genes *Me3*, *Me1* and *N*, routinely used in breeding pepper cultivars resistant towards *M. arenaria*, *M. incognita* and *M. javanica*, the two genes *Me3* and *Me1* are stable at high temperature (Djian-Caporalino *et al.*, 1999), whereas the efficacy of *N* was partially lost at 32°C (Thies and Fery, 1998). Furthermore, *Me3* triggers an early hypersensitive

response in the root epidermis at the nematode penetration site, whereas *Me1* triggers a later response in the root vascular cylinder (Bleve-Zacheo *et al.*, 1998; Pegard *et al.*, 2005).

The small-fruited hot peppers *Capsicum frutescens* cv. *longum* are resistant to the major species of root knot, but not *M. hapla* (Johnson, 1998). A number of root knot-resistant cultivars of bell pepper, *C. frutescens*, have been released in the USA. They are homozygous for the dominant *N* resistant gene toward *M. incognita*, *M. javanica* and races 1 and 2 of *M. arenaria* (Fery *et al.*, 1998; Thies and Fery, 2000a). The cultivar 'Charleston Belle' has been field tested with good results (Thies *et al.*, 2004), and resistance has been shown to hold up well under high soil temperatures that often negatively affect nematode resistance in other horticultural crops (Thies and Fery, 2000b).

Resistance has also been found in aubergine, where it was originally detected in *Solanum sisymbriifolium*, a close relative. Several wild species of *Cucumis* with resistance to root knot have also been detected (Fassuliotis, 1979). However, genetic barriers make it extremely difficult to introduce the resistance into cultivated species. Modern molecular techniques such as protoplast culture and somatic hybridization may make it possible to create viable hybrids, and attempts are being made to develop interspecific hybrids (Starr *et al.*, 2003).

To date, little progress has been made in developing resistance to root knot in the Cucurbitaceae. Liu *et al.* (2015) reported that the wild *Cucumis* species, *Cucumis pustulatus* f., has promising potential as rootstock for cucumber, melon and watermelon. Overall, most cucumber varieties seem to be more resistant to *M. hapla* and *M. arenaria* than to *M. incognita* or *M. javanica* (Johnson, 1998).

Resistant oil radish genotypes of *R. sativus*, a green manure crop used effectively to control sugarbeet cyst nematodes, are effective in reducing *M. hapla* and *M. incognita* densities in greenhouse and microplot tests (Bünthe and Müller, 1996; Bünthe *et al.*, 1997).

Lists of plants reported to be resistant to nematodes in general (Armstrong and Jensen, 1978) and crop cultivars resistant to species of *Meloidogyne* (Sasser and Kirby, 1979; Netscher and Sikora, 1990; Whitehead, 1998; Williamson and Roberts, 2009) have been compiled elsewhere.

Lists of cultivars resistant to root knot nematodes, however, should be used with caution, because some of the cultivar reactions are often based on a limited number of field observations. Such tests are also not a guarantee that a cultivar is resistant to all populations of *Meloidogyne*.

Currently, nematode resistance in vegetable crops is based on single, major resistance genes. As those genes are rare and their efficacy threatened by virulent populations overcoming this resistance, new concepts are needed. One option is to combine several resistant genes or to go for a more durable quantitative, often partial, resistance. However, the genetic determinants of the partial resistance are, in most cases, unknown. Therefore, quantitative trait loci (QTL) analysis has to be performed to define those traits as a prerequisite for further use in breeding programmes (Barbary *et al.*, 2016).

With the ongoing efforts of sequencing complete plant genomes, nematode resistance genes have been, often unexpectedly, detected in crop species, raising questions such as what are they doing, why some seem to be dormant and how can they be reactivated? Marker-assisted selection helps to detect resistant individuals in segregating populations, and modern genome-editing techniques such as the CRISPR Cas9 (clustered regularly interspaced short palindromic repeats) are already used to improve resistances in vegetables towards plant pathogens (Xiong *et al.*, 2015). It seems that the future of resistance breeding has just begun. Hopefully, interest by scientists and breeders will include resistance against *Meloidogyne* in vegetable crops.

GRAFTING: where resistant cultivars are not available, grafting of commercially valuable crop cultivars on to nematode-resistant rootstocks is an option. Grafting has been practised since the 1920s in Japan and Korea, and has become highly regarded in vegetable production where pest pressures are high. In Japan, 59% of the cucumber, tomato, aubergine, watermelon and melon grown in protected cultivation are grafted on to various rootstocks for increased vigour and tolerance or resistance to pests and diseases, including *Meloidogyne*. Grafting robots have been developed to produce grafted plugs in nurseries (Oda, 1999). However, resistant rootstocks are similarly prone to nematode pathotypes as resistant cultivars, and therefore resistance

management is equally important. Variable response to *Meloidogyne* spp. isolates and the selection of virulent populations has occurred.

Solanum torvum, which has a high level of resistance to *M. incognita* and *M. arenaria* and is a poor host for *M. javanica*, has been used successfully as a rootstock for aubergine (Dunay and Dalmasso, 1985). When the shoots (scions) of aubergine were grafted on to the rootstocks of *S. torvum*, *Solanum aethiopicum*, *S. sisymbriifolium*, *Cyphomandra betacea* (tamarillos), tomato line NR 62 or tomato cv. Gallo de Castellana and compared with plants maintained on their own roots, the *Solanum* and tomato rootstocks all reduced plant susceptibility to *Meloidogyne*, with *S. torvum* the best combination for both control and yield (Porcelli *et al.*, 1990). Additional trials showed that aubergine grafted on to *S. torvum* rootstocks having resistance to root knot and soil-borne pathogens could compete with soil fumigation, regarding both control and yield increases (Morra *et al.*, 1992). Of the seven wild species of *Solanum* tested, three were found resistant to *M. incognita*: *S. sisymbriifolium*, *S. torvum* and *Solanum toxicarium* (Mian *et al.*, 1995a). These species, when used as rootstocks, not only reduced galling on tomato but also reduced bacterial wilt of aubergine caused by *R. solanacearum* (Mian *et al.*, 1995b).

Granges and Leger (1996) showed that when susceptible tomatoes were grafted on to rootstocks having resistance to species of *Meloidogyne* and various root pathogens, yield increased 50 and 30% at the beginning and end of harvest when compared with the non-grafted plants, respectively. Additional soil steaming did not increase the productivity of grafted plants. The highest profit margin was obtained when plants were grafted with two stems per nematode-resistant rootstock and planted at half the standard density. Susceptible tomato cultivars grafted on to the nematode-resistant rootstocks also produced significant yield increases and *M. incognita* control (Morra *et al.*, 1997). In tests conducted in Spain, grafted tomato held an intermediate place in both the level of control and the effect on yield increase between the resistant and the susceptible cultivar, which varied among rootstocks (Verdejo-Lucas *et al.*, 2009). The phenotypic resistance response of rootstocks to several populations of *M. javanica*, *M. incognita* and *M. arenaria* ranged from highly resistant to susceptible and was

nematode-isolate specific (Augustin *et al.*, 2002; Cortada *et al.*, 2008, 2009), which was linked to the presence of several *Mi*-homologues on the background of these hybrid (*Solanum* spp. $\times$ *S. lycopersicum*) tomato rootstocks. The durability of the *Mi*-gene-mediated resistance was lower in tomato rootstocks than on resistant tomato cultivars, as *Mi*-virulent *Meloidogyne* populations were selected after repeated cultivation of tomato rootstocks (Verdejo-Lucas *et al.*, 2009). This was attributed to the frequency of *Avr/avr* alleles in the genetic pool on the nematode population, the genetic background of the resistant rootstock and the frequency of cropping. Rootstock resistance was maintained even if temperatures peaked above 28°C (Verdejo-Lucas *et al.*, 2009).

RESISTANCE MANAGEMENT: in most cases, virulent individuals will have a selective advantage over avirulent individuals when a resistant cultivar is grown. This advantage is lost in the presence of a susceptible cultivar, and virulent population development will decline. Therefore, growing susceptible cultivars within a rotation can delay or prevent even the build-up of virulent populations. Resistance management strategies need to be an integral part of any vegetable production system wherever cultivars with single resistance genes are grown.

In southern USA, double cropping susceptible cucumbers using cucumber transplants versus direct sowing, when grown after a nematode-resistant tomato crop, was effective in improving cucumber yields in *M. incognita*-infested soils (Hanna *et al.*, 1994, 1996). This approach was more effective than a nematicide applied through drip irrigation for managing *M. incognita* (Colyer *et al.*, 1998). Similar results were obtained by double cropping cucurbit crops after the root knot-resistant bell pepper 'Charleston Belle' (Thies *et al.*, 2004). Cucumber yields were 87% heavier and the number of fruit 85% higher when planted after the resistant pepper cultivar. Squash yield increased 55% and the number of fruit 50% over the fields previously planted to susceptible pepper. Cropping systems of this nature allow the production of multiple cycles of high-value crops with simultaneous protection of resistant germplasm.

WEED CONTROL: weeds can be good hosts for root knot nematodes (Ornat and Sorribas, 2008;

Rich *et al.*, 2009; Ntidi *et al.*, 2012, 2016) (Table 10.8; Fig. 10.11). If weed hosts are not properly controlled, they can sustain root knot populations even when cropping non-hosts in a rotation. Therefore, proper weed control contributes greatly to nematode management and effective crop improvement.

A unique nematode management approach that takes advantage of weed growth has been developed in Costa Rica. All weeds, hosts and non-hosts that grow in the rainy season before the next major vegetable crop are incorporated into the soil, drip irrigation added and the beds mulched with plastic. This combination leads to optimum moisture, high temperatures and a biofumigation that provides effective weed control and root knot management simultaneously. Planting is then conducted through the mulch into the soil (R. Garron, Costa Rica, 2004, personal communication).

Chemical control

Chemical control was, and still remains, the mainstay for *Meloidogyne* control in intensive vegetable production systems (Nyczepir and Thomas, 2009), attracting 38% of the global nematicide market (Haydock *et al.*, 2006). Across all crops, *Meloidogyne* is the predominant group, targeting 48% of the global nematicide market.

Nematicides used against root knot nematodes are either fumigants, which are usually liquids and enter the soil water solution from a gas phase, or non-fumigants that are granular or liquid compounds, which are water soluble. In most cases, the fumigants are broad-spectrum contact nematicides effective against juveniles and eggs, as well as other pests, diseases or weeds. Non-fumigant nematicides have either contact or nematostatic and systemic activity against nematodes, and often against insects, but are not effective against eggs and, in most cases, do not kill the juveniles at the recommended dosages. In most cases, the mechanism of action is associated with the suppression of nematode mobility during the period when adequate concentrations are in the soil solution. Non-fumigants give the plant a 'head start' by delaying nematode penetration during the highly sensitive seedling or post-transplant stage of plant development. There are a number of

Table 10.8. Comparison of selected weed hosts of important root knot nematodes attacking vegetables. (From: Goodey *et al.*, 1965; Fernández *et al.*, 1993; Barcelo *et al.*, 1997; Brito *et al.*, 2004; Ornat and Sorribas, 2008.)

Weed	<i>Meloidogyne</i> species					
	<i>incognita</i>	<i>javanica</i>	<i>arenaria</i>	<i>hapla</i>	<i>chitwoodi</i>	<i>enterolobii</i>
<i>Amaranthus albus</i>	•	•				
<i>Amaranthus retroflexus</i>	•	•	•	•		
<i>Ajuga reptans</i>				•		•
<i>Anthemis arvensis</i>				•	•	
<i>Atriplex papula</i>	•					
<i>Capsella bursa-pastoris</i>	•	•	•	•		
<i>Chenopodium album</i>	•	•	•	•		
<i>Cirsium arvense</i>	•	•		•	•	
<i>Clerodendrum ugandense</i>	•					•
<i>Convolvulus arvensis</i>	•	•				
<i>Cyperus rotundus</i>	•	•				
<i>Digitaria sanguinalis</i>	•	•	•			
<i>Erigeron</i> spp.	•	•		•		
<i>Erodium cicutarium</i>	•			•		
<i>Galinsoga ciliata</i>				•		
<i>Galinsoga parviflora</i>	•	•	•	•	•	
<i>Lamium amplexicaule</i>	•			•	•	
<i>Medicago arabica</i>		•	•			
<i>Poa annua</i>	•		•			
<i>Polygonum persicaria</i>	•			•		
<i>Portulaca oleracea</i>	•	•	•	•		
<i>Rumex crispus</i>		•	•			
<i>Senecio vulgaris</i>	•		•	•		
<i>Setaria verticillata</i>	•	•	•			
<i>Solanum nigrum</i>	•	•		•	•	
<i>Sonchus oleraceus</i>	•	•	•	•		
<i>Sonchus tenerrimus</i>	•	•	•			
<i>Stellaria media</i>	•	•	•	•		
<i>Taraxacum officinale</i>	•			•	•	
<i>Tibouchina elegans</i>						•

sources that give excellent reviews on the use of the most common fumigant and non-fumigant nematicides for a broad array of nematodes and crops, and they should be consulted for more detail (Haydock *et al.*, 2006; Nyczepir and Thomas, 2009). Although nematicides in general are key components of intensive vegetable production, their use by smallholder farmers is thought to be too expensive. However, in the intensive peri-urban and urban systems especially, synthetic nematicides are used regularly, but often to the point of overuse and misuse due to a lack of understanding and/or awareness of nematode issues (Neuenschwander, 2004; Ntow *et al.*, 2006; James *et al.*, 2010).

FUMIGANT NEMATICIDES: fumigant nematicides include compounds containing halogenated hydrocarbons (e.g. chloropicrin, methyl iodide, 1,3-dichloropropene) or those that discharge carbon disulfide (e.g. tetrathiocarbonate) or methyl isothiocyanate (e.g. metam sodium, dazomet). They are generally more effective in controlling root knot nematodes and in increasing crop yield than are non-fumigant nematicides. Except for 1,3-dichloropropene being specific against nematodes, most other fumigants have a broader spectrum, also targeting insects, fungi and weeds (Hutchinson *et al.*, 1999). Most of the fumigant nematicides have been shown to be highly effective in control programmes designed



Fig. 10.11. Root galling on *Artemisia* in a vegetable field caused by *Meloidogyne incognita*. (Photograph courtesy of E. Fernández.)

to reduce *Meloidogyne* losses in vegetables (Lamberti, 1979b; Whitehead, 1998; Nyczepir and Thomas, 2009). Fumigants are typically applied to the soil. The exception is metham sodium, which can be applied by drip irrigation or even centre-pivot overhead systems. In some growing areas, fumigants are applied under plastic mulch and the vegetables are planted through the mulch, usually in raised beds.

NON-FUMIGANT NEMATOCIDES: non-fumigants are formulated as either granular or liquid materials, and can have contact and/or systemic capacities. They include products such as oxamyl, ethoprop/ethoprophos, fenamiphos, carbofuran, cadusafos, fluopyram, fosthiazate, phorate, thiodicarb and terbufos, which to some degree are all effective against *Meloidogyne* (Cadet, 1990; Basile *et al.*, 1993; Lamberti *et al.*, 1993; Philis, 1994; Verma *et al.*, 1994; Sasanelli *et al.*, 1996; Whitehead, 1998). In general, these nematicides either block the neuron transmitter acetylcholinesterase or prevent the formation of adenosine triphosphate, the cell energy source, which in both cases causes paralysis of the nematode. Most non-fumigant nematicides, however, are highly toxic to humans and the environment, with some already withdrawn from use. Growers need to be aware of proper handling and application, and that residues may persist in the harvested crop when applied improperly.

Non-fumigant nematicides are often not as effective as fumigants in increasing yields, because they do not have broad-spectrum activity and in most cases only inactivate nematodes for short periods. Therefore, repeated applications are needed in multiple-cropping vegetable systems. This is often uneconomical, environmentally questionable and can lead to biodegradation over time (Mojtahedi *et al.*, 1991; Stirling *et al.*, 1992).

Granular nematicides are either applied broadcasted over the soil surface and incorporated into the soil before planting or banded into or over the plant furrow. Liquid formulations allow application by surface and drip irrigation (Whitehead, 1998), with the latter of extreme importance to vegetable production. Application through drip irrigation places the material directly in the rhizosphere and allows splitting or extending application over specific time intervals to coincide with optimum control. For example, oxamyl applied to tomato by drip irrigation was more effective in controlling *Meloidogyne* than granular nematicides applied at transplanting (Philis, 1994; Russo *et al.*, 2003). Dip treatment or treatment of transplants in nurseries (Ahuja, 1978; Mateille and Netscher, 1985; Jansson and Rabatin, 1998) have also been effective. For example, Siddiqui *et al.* (1998) showed that dip treatment of seedlings of aubergine and tomato with fenamiphos significantly reduced *M. incognita* galling.

Efforts are being made to develop formulations that allow seed treatment for nematode control that would reduce greatly the dose needed on a per hectare basis, reduce the environmental impact and reduce crop residues. In many short-cycle vegetable crops and in crops with a taproot that may only need protection of 4–5 weeks, this could be an important treatment form. Townshead (1990) showed that seed of carrot and tomato coated with oxamyl resulted in reduced galling by *M. hapla*. The treatment of bottle and bitter gourd with carbofuran, fenamiphos or phorate reduced *M. incognita* and increased yields (Siddiqui *et al.*, 1993).

So far, nematicidal seed treatments based on avermectin and terbufos have been developed for major field crops such as maize, soybean and cotton, but could be of potential use in vegetables. Avermectins are macrocyclic lactones produced by the actinomycete *Streptomyces avermitilis* that have broad-spectrum nematicidal

activity (Cayrol *et al.*, 1993). They are active against root knot as root dips and as seed treatments on a number of crops (Jansson and Rabatin, 1998). Effective control of *M. hapla* was obtained with low doses of abamectin as a seed treatment on pelleted and non-pelleted seeds of tomato (Abawi *et al.*, 2003), with root galling and the number of eggs/g root significantly reduced after 6 weeks. Abamectin was also effective in controlling *M. incognita* as a seed treatment of cucumber (Becker *et al.*, 2003; Becker and Hofer, 2004). Meanwhile, fluopyram, a new-generation product delivers wide spectrum, long-lasting control of nematodes.

In conclusion, fumigant and non-fumigant nematicides are effective components of management programmes for root knot. They will remain important in cropping situations where alternatives are not available or not effective. With the withdrawal from use of a number of these, such as methyl bromide, new nematicides with lower mammalian toxicity, which are more environmentally sensitive, are being sought and are needed desperately. In some cases, these are under evaluation, as are more novel mechanisms for reducing the dosage levels through innovative delivery techniques, such as seed or seedling treatments or through drip irrigation, for example.

Biological control

Biological means for *Meloidogyne* control in vegetables can follow several approaches: (i) the inundative application of bacterial and fungal antagonists that infect eggs, juveniles or adults in the soil or on the root surface (= biological nematicides) (Jatala, 1986; Kerry, 1987; Kerry and Evans, 1996; Atkins *et al.*, 2003; Stirling, 2014); (ii) field inoculation and management with the obligate bacterial parasite *Pasteuria penetrans* (Oostendorp *et al.*, 1991; Trivino and Gowen, 1996; Gowen *et al.*, 1998; Stirling, 2014); (iii) the promotion of the naturally occurring antagonistic potential in soils with amendments or crop rotation (Sieverding, 1991; Sikora, 1992; Pyrowolakis *et al.*, 2002); (iv) the biological enhancement of transplants or planting material with plant health-promoting rhizosphere- or endorhiza-associated bacteria or fungi (Sikora, 1992, 1997; Sikora and Hoffmann-Hergarten, 1993; Hallmann and Sikora, 1994a,b; Sikora *et al.*, 2003).

Recent success in the development of bacterial and fungal antagonists has coincided with significant advances in fermentation and formulation technology (Silman *et al.*, 1993; Lüth, 2000; Kiewnick, 2001; Lüth and Eiben, 2003). This has led to the development of several antagonists into biological nematicides (Hallmann *et al.*, 2009). Some of the better-studied antagonists are the fungi *Purpureocillium lilacinum*, *P. chlamydosporia* and *Trichoderma* spp., as well as the bacteria *Bacillus firmus* and *P. penetrans*, which are presented below.

P. lilacinum (syn. *Paecilomyces lilacinus*) is a well-known egg pathogen. Its potential to control root knot in the field has been reported on vegetables in a number of countries (Davide and Zorilla, 1983; Cabanillas and Barker, 1989; Lara *et al.*, 1996; Aceret *et al.*, 1999; Kiewnick and Sikora, 2003; Singh *et al.*, 2013). The fungus mainly infects the eggs of *Meloidogyne*. In addition, spores of the fungus adhere to the cuticle of juveniles, where they germinate and penetrate the cuticle of the nematode. Finally, hyphae of the fungus can enter the nematode through body openings, such as the anus and vulva, to kill fully developed females. Due to its broad host spectrum and high control potential, the fungus was developed as a biological nematicide that was marketed by several companies under different trade names for use in warmer climates worldwide. An optimum application scenario in the case of BioAct®WP (wetttable powder) containing 1×10^{11} spores of *P. lilacinum* strain 251 per gram for root knot control on vegetables would consist of three steps: (i) pre-plant soil treatment 14 days before planting at 0.02 g/plant or maximum 400 g/ha; (ii) seedling treatment just before planting at 0.001 g/100 ml potting soil; and (iii) post-planting drench 6 weeks after planting at 0.02 g/plant or maximum 400 g/ha. Field application can be conducted by spraying or drip irrigation as long as a good distribution of the product in the top 20 cm of soil is assured.

Instead of applying conidia, ZhangYong *et al.* (2016) tested the potential of microsclerotia of *P. lilacinum* against *M. incognita*. They observed excellent nematophagous ability and a greater thermotolerance and UV-B radiation tolerance compared to conidia, and suggested that the use of microsclerotia might be superior to conidia for biocontrol products. However, maximum yield of

microsclerotia during liquid fermentation was 1000-fold lower than for conidia, affecting cost-effectiveness.

P. chlamydosporia has been studied extensively both in the laboratory and under field conditions for root knot control (De Leij and Kerry, 1991; Kerry, 2000, 2001; Atkins *et al.*, 2003; Hildalgo-Diaz and Kerry, 2008; Manzanilla-López *et al.*, 2013). The fungus colonizes the rhizosphere of host plants and efficiently parasitizes root knot eggs once they are exposed on the root surface, but does not parasitize juvenile stages and therefore will not reduce initial plant infection. However, once established in the rhizosphere, *P. chlamydosporia* provides sufficient control. Bourne and Kerry (1999) and Bourne (2001) demonstrated that the application of *P. chlamydosporia* in a rotation of less susceptible crops, such as kale, beans and cabbage, led to large reductions in root knot densities in the subsequent tomato crop, because egg masses were more exposed to the fungus on the rhizosphere of these crops.

A chlamydospore-based product of *P. chlamydosporia* (KlamiC®) is used successfully in Cuba in peri-urban agriculture (Fernández-Larrea, 2012). Various strains of the fungus are also commercially available in other countries (Monzanilla-López *et al.*, 2013). Fungal chlamydospores are commonly produced by solid-state fermentation using different substrates, resulting in approximately 10⁶ chlamydospores/g of substrate. Application rates range from 30 to 400 kg/ha (Stirling and Smith, 1998; Verdejo-Lucas *et al.*, 2003).

T. harzianum, which is known to be effective against fungal diseases, also has activity toward root knot nematodes. When applied in maize, *T. harzianum* and *Trichoderma koningii* increased plant growth and reduced *M. arenaria* on maize under controlled conditions (Windham *et al.*, 1989). Control of root knot with an Indian strain of *T. harzianum* was enhanced by adding the antagonist to soil amended with neem cake (Rao *et al.*, 1997). Both *T. harzianum* and *Trichoderma lignorum* increased plant growth and reduced *M. javanica* galling on tomato and aubergine in soil treated with the fungi 18 days prior to planting in greenhouse tests (Spiegel and Chet, 1998; Sharon *et al.*, 2001). Single treatments of *T. harzianum* and *Trichoderma viride* were effective at initial low root knot densities in one-cycle

vegetable crops grown in organoponics (Pérez, 2001). Radwan *et al.* (2012), testing two commercial products of *Trichoderma album* (Biozeid®) and *T. harzianum* (Plant Gard®), found a similar control of *M. incognita* infecting tomato, as with oxamyl and carbofuran. In Benin, Affokpon *et al.* (2011b) evaluated 17 populations of indigenous species isolated from farmers' vegetable plots and showed that *Trichoderma asperellum* T-16 suppressed second-stage juvenile densities in roots by up to 80%, increasing tomato yields by over 30%, while *Trichoderma brevicompactum* T-3 suppressed egg production by as much as 86%. This demonstrates to some extent the potential of such options for root knot management.

B. firmus is a naturally occurring soil bacterium that has been developed commercially for controlling *Meloidogyne*, such as *B. firmus* strain GB-3, marketed as BioNem® WP in Israel (Keren-Zur *et al.*, 2000). The product provided good suppression of *Meloidogyne* spp. within 2 months of transplanting cucumbers, which persisted through the end of the experiments (Giannakou *et al.*, 2004). However, the control provided by *B. firmus* GB-126 was less effective than the soil fumigant, dazomet. In field trials with tomato, BioNem® applied at 200 and 400 kg/ha was effective in reducing root galling caused by *M. incognita* by 75–84% over the non-treated control (Terefe *et al.*, 2009). Similarly, *B. firmus* I-1582 (Flocter®) provided considerable control of a broad spectrum of plant parasitic nematodes, including *Meloidogyne*, on vegetables (Tiraferri *et al.*, 2012).

P. penetrans is an obligate parasite of *Meloidogyne* (Birchfield and Antonopoulos, 1976; Chen and Dickson, 1998; Stirling, 2014). Endospores of *P. penetrans* can resist both drought and exposure to non-fumigant nematicides (Mankau and Prasad, 1972). Stirling and Wachtel (1980) produced large numbers of spores by inoculating tomato with infected *Meloidogyne* juveniles. Dried tomato roots were then milled into a powder, which contained *Pasteuria* spores, a method that can be adapted to produce inoculum for small growers. The parasite can also be increased in root knot-infested fields by growing tolerant or moderately resistant crops (Oostendorp *et al.*, 1991; Gowen *et al.*, 1998; Gowen and Pembroke, 2004). Growing root knot host plants over three cycles increased spore numbers in the soil that prevented root knot invasion (Melki

et al., 1998). The parasite tends to be more effective in warm soils and soils low in organic matter, which characterizes most tropical soils where root knot is a problem. Solarization and/or soil amendments may reduce the efficacy of *P. penetrans* (Freitas *et al.*, 2000a). Conversely, chloropicrin, found in many fumigant nematocides, was shown to have bactericidal effects on *P. penetrans* (Freitas *et al.*, 2000b), as did fumigation with 1,3-dichloropropene (Timper *et al.*, 2016). Metam sodium, on the other hand, did not affect the parasite adversely, which is important in intensive production systems where this fumigant is used. Combining the parasite with plant resistance, oxamyl and solarization also reduces root knot (Tzortzakakis and Gowen, 1994).

The 'antagonistic potential' of a soil and its management has been considered a means of reducing the impact of root knot and other nematodes (Sikora, 1990, 1992; Stirling, 2014). The specific components of this potential can be measured and changes monitored using simple bioassays (Rodríguez-Kábana *et al.*, 1994; Pyrowolakis *et al.*, 2002). Managing the antagonistic potential to improve suppressiveness requires knowledge of the microbial communities (Vilich and Sikora, 1998) and, more specifically, the antagonists conducive to management. Arbuscular mycorrhizal fungi are management-sensitive antagonists that are present in all soils and that react favourably to certain crop rotations (Smith, 1987; Sieverding, 1991; Sikora, 1995). A number of rotation crops such as vetch, clover, maize, bahiagrass and pearl millet, which are poor or non-hosts of root knot and decrease nematodes in rotations, also increase mycorrhizal densities in soils, which can then have positive effects on the following root knot-susceptible crop (Sumner *et al.*, 1999; Jothi and Rajeswari, 2001). It should be noted that crop rotation may also favour non-effective mycorrhizal species (Sieverding, 1991). The overall antagonistic potential of a soil can be managed, for example, using organic amendments and green manures of various forms (Singh and Sitaramaiah, 1966, 1967; Sikora *et al.*, 1973; Hildalgo-Diaz and Kerry, 2008). Amendments stimulate many antagonists and also lead to the production of toxic metabolites and/or biofumigants that together are responsible for the effectiveness of this management practice.

Biological enhancement of seeds and vegetable transplants with antagonistic microorganisms, e.g. arbuscular mycorrhizal fungi, mutualistic fungal endophytes, plant health-promoting rhizosphere or mutualistic endophytic bacteria, can increase plant resistance and/or tolerance to root knot infection (Sikora, 1990, 1997; Sikora and Hoffmann-Hergarten, 1993; Schuster *et al.*, 1995; Hallmann, 2001; Hallmann *et al.*, 2009).

Biological enhancement has been attained using antagonistic rhizobacteria as seed dressings (Sikora, 1988; Oostendorp and Sikora, 1989) and through application by drip irrigation systems (Zavaleta-Mejia and Van Gundy, 1982). Tomato and pepper transplant substrate treated with formulations of plant growth-promoting rhizobacteria caused highly significant increases in tomato and pepper growth, vigour and survival in the field, with some formulations reducing the numbers of root knot galls on pepper (Kokalis-Burelle *et al.*, 2002). Rhizobacteria added to tomato 'seedling' trays under commercial nursery conditions caused significant reductions in root knot galling and increased tomato growth and yield (Reddy *et al.*, 2000). Some rhizobacteria, especially those that grow endophytically, have been shown to induce resistance (Hasky-Günther *et al.*, 1998). Some isolates also have activity toward root knot and simultaneous activity toward *Fusarium* wilt of tomato (Hauschild *et al.*, 2000). Endophytic bacteria have been shown to reduce root knot infection significantly (Munif *et al.*, 2000) and induce systemic resistance in tomato (Munif *et al.*, 2001). Mahdy *et al.* (2000, 2001) also demonstrated significant levels of protection against species of *Meloidogyne* using rhizosphere and endophytic bacteria.

The enhancement of plants with arbuscular mycorrhizal fungi, apart from increasing plants' access to nutrients, reduces the penetration and development of a number of root knot nematodes on a range of vegetable crops (Sikora, 1978, 1995; Hussey and Roncadori, 1982; Smith, 1987; Affokpon *et al.*, 2011a). An important strategy for effective control in vegetable crops is the enhancement of seedlings to attain high mycorrhizal root colonization densities at transplanting. Mycorrhizal inoculum is commercially available for this purpose in many countries. Combining mycorrhizal fungi with plant health-promoting rhizobacteria during

seedling development led to increased mycorrhiza colonization and root knot control in tomato at transplanting (Reimann and Sikora, 2003).

Endophytic fungi are prime antagonists for use in the biological enhancement of transplants for root knot control in one-cycle cropping systems. Hallmann and Sikora (1994a,b) showed that *M. incognita* galling was reduced by 50% in tomato inoculated with a mutualistic fungal endophyte. Root knot control with a non-pathogenic *F. oxysporum* isolate was significantly higher than with *Trichoderma* species in greenhouse tomato (A.A. Dababat and R.A. Sikora, 2004, unpublished data). Mutualistic endophytic fungi have an advantage over arbuscular mycorrhizae in that they can be produced by liquid- or solid-state fermentation. They actively colonize the growth substrate as well as the endorhiza of the root occupied by the nematode (Schouten, 2016). A product based on *T. asperellum* (RealTrichoderm®) when applied as a drench to seedlings provided significant root knot control to tomato and pepper under controlled conditions and in the field in Kenya, Tanzania and Uganda (Coyne, IITA, Nairobi, 2017, personal communication). In Benin, indigenous populations of nematophagous fungi were assessed for their ability to also colonize tomato plants endophytically, including *Trichoderma* species, *P. chlamydosporia* and a population of *Aspergillus allahabadii*, all of which provided positive results for protection against root knot nematodes (Affokpon, 2011).

The treatment of fumigated, biofumigated or solarized soil with biologically enhanced transplants would increase overall control, due to the lack of competitive microbial activity in this soil. To be effective, however, biological enhancement requires the existence of either commercial biocontrol products, which are accessible by small or large commercial nursery production units that supply enhanced seedlings to growers. In some countries, for example in Cuba, antagonists are produced centrally on a large scale (Fernández *et al.*, 2000; Pérez, 2001; Hildalgo-Diaz and Kerry, 2008), and enhanced seedlings are the result of inoculation in organoponic production. In many countries, large commercial growers produce their own high-quality seedlings, and in some instances large food chains supply contract

growers with healthy seedlings. These could be inoculated with antagonists prior to delivery to growers, to increase yield and reduce reliance on pesticides.

Summary of management measures

Based on the above, we consider the following aspects important for the management of *Meloidogyne* for both field and protected cultivation of vegetables:

- Prevent introduction through good quarantine.
- Build protected cultivation structures on root knot-free land.
- Utilize nematode-free growth substrates for seedling production.
- Plant certified root knot-free transplants.
- Introduce antagonists into the seedling production system.
- Always view previously infested land as infested.
- Develop farmer-based root gall monitoring protocols to estimate infestations.
- Design rotations that prevent the build-up of high nematode densities.
- Use nematicides judiciously based on monitoring previous crops.
- Take advantage of resistant cultivars when available.
- Monitor soil temperature to prevent breakdown of *Mi* gene resistance.
- Rotate resistant and susceptible cultivars to prevent resistance-breaking pathotypes.
- Challenge all new crops with local populations to assess host status.
- Use root grafting of resistant rootstocks if economical.
- Tolerant rootstocks should be used if adaptable where resistance is lacking.
- Use paddy rice or controlled flooding for control.
- Destroy galled roots after harvest.
- Incorporate nematode desiccation through tillage-supported soil drying.
- Time planting for cooler periods to reduce infection.
- Biofumigate with incorporated organic amendments.
- Use organic matter to stimulate antagonistic potential.

- Trap crop if exact timing for crop destruction can be ensured.
- Introduce solarization in solar energy-rich regions.

Methods of diagnosis

The presence of *Meloidogyne* can be verified easily by observing root galls on a susceptible host. Crops or weeds can be uprooted systematically

and scored for severity of root galling, providing an accurate estimation of the severity and the distribution of *Meloidogyne* within a field. A number of different root knot indices have been proposed (Zeck, 1971; Barker, 1985), of which the index by Bridge and Page (1980) provides a reliable index for tomato based on a scale of 1–10 (Fig. 10.12), while Coyne *et al.* (2014) provides a photographic basis of different galling styles using a scale of 1–5. Root gall index and

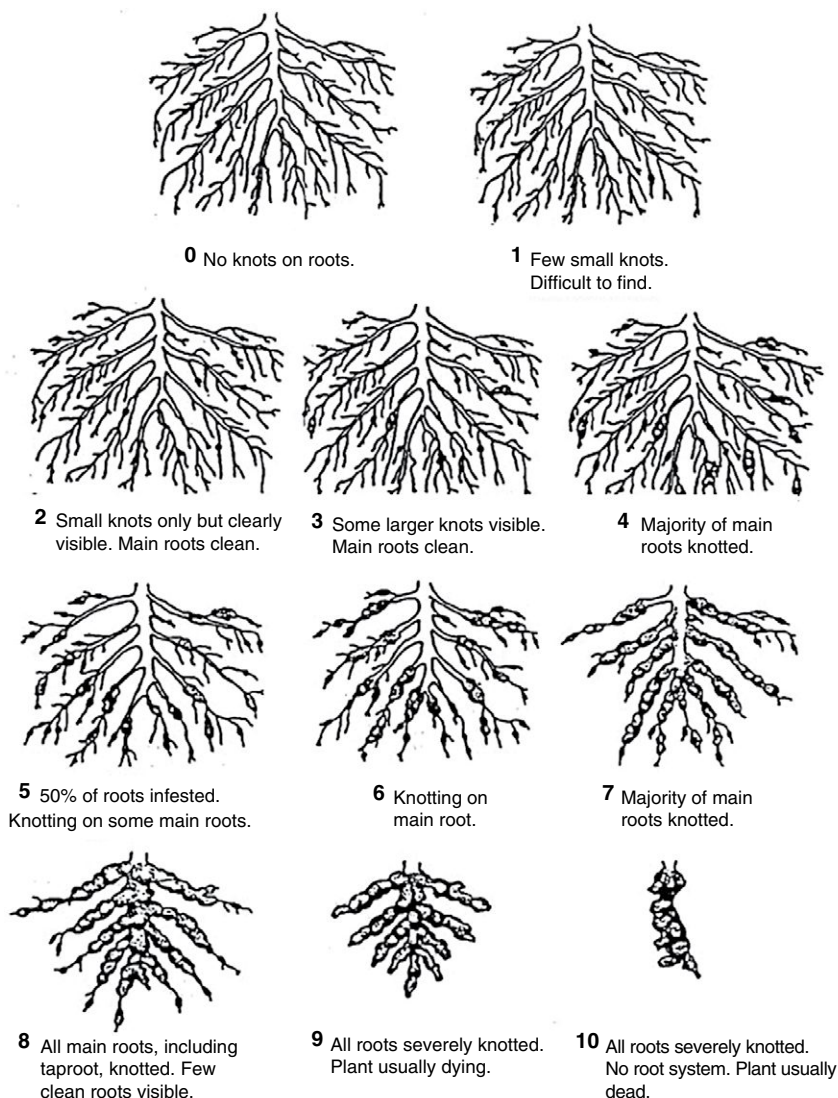


Fig. 10.12. Root galling rating scheme for the evaluation of *Meloidogyne* infestation. (From Bridge and Page, 1980.)

yield loss have a linear relationship, which varies in degree as to crop and environmental conditions, allowing a rough but reliable estimate of the crop damage (Barker *et al.*, 1981).

Bioassay techniques, in which susceptible plants (e.g. lettuce, cucumber, tomato) growing in the field or in pots containing a representative field sample are uprooted and examined for the presence of galls after a period of 3–6 weeks, constitute a means to evaluate the presence of infestations with greater accuracy than soil analysis (McSorley and Parrado, 1983).

However, certain conditions require a more thorough evaluation of the *Meloidogyne* situation in the field, such as for management decisions based on threshold levels requiring accurate densities or the selection of non-host crops requiring species identification. Any field sampling should be representative considering the scattered or clustered distribution of *Meloidogyne*. See Duncan and Phillips (2009) and Been and Schomaker (2013) for more information on nematode distribution in the field. Furthermore, the majority of *Meloidogyne* may be present in the form of eggs or egg masses, which are not covered by standard extraction methods. However, techniques have been developed based on the fact that the egg masses remain intact in the soil either free or attached to host roots or root fragments (Dickson and Strubel, 1965; Byrd *et al.*, 1972; Gooris and d'Herde, 1972). After separating the organic matter from the soil using sieving or elutriation techniques, eggs are liberated from egg masses either chemically (Byrd *et al.*, 1972) or mechanically (Gooris and d'Herde, 1972). Egg masses can also occur in the mineral fraction of the soil, while Demeure and Netscher (1973) suggested incubation of this fraction. Incubation of the mineral as well as organic fraction for 2–4 weeks at room temperature and counting hatched juveniles following extraction by Baermann (see Chapter 4, this volume) is routine to provide quantitative data on root knot densities.

Rotylenchulus

The reniform nematode *R. reniformis* is the second most important nematode affecting vegetables. The nematode attacks over 100 plant species, including many vegetable crops, and is a limiting

factor in vegetable production, but is often neglected or overlooked, especially where it occurs concomitantly with *Meloidogyne* (Roy and Mukhopadhyay, 2005, 2011; Roy *et al.*, 2007; Marwoto, 2010). The nematode has been detected in over 36 countries (Heald and Thames, 1982). It has been recorded in Hawaii, where it was first described (Linford and Oliviera, 1940), in southern USA, Mexico, the Caribbean, South America, the Middle East, most of Africa, India, South-east Asia and the Pacific.

Symptoms of damage

Above-ground symptoms include stunting and leaf curling (Singh and Khera, 1979). Root necrosis and cortical necrosis have been observed following infection. Cantaloupe growing in heavily infested soil was badly stunted and yields were greatly reduced (Heald, 1975). Leaf chlorosis may be evident (Bridge, 1983). Females and their adhering egg masses can be observed easily under the dissecting microscope. Soil adhering to the gelatinous egg masses often gives them a dark appearance, aiding detection.

Biology

Immature females penetrate the root and become sedentary. Galls are not produced. The life cycle is completed on okra in 24–29 days (Sivakumar and Seshadri, 1971). The existence of amphimictic and parthenogenetic races of *R. reniformis* has been demonstrated by Hirschmann and Triantaphyllou (1964).

The reniform nematode can survive in moist soil in the absence of hosts for 7 months, and for 6 months in dry soil. After 4 months, 84% of the nematodes were still alive (Sivakumar and Seshadri, 1979), but survived 29 months in the absence of host plants (Stoyanov, 1971).

The intensity of Brinjal mosaic virus and Okra yellow vein mosaic virus was promoted on plants parasitized by *R. reniformis* (Sivakumar and Meerzainuddeen, 1973; Naqvi and Alam, 1975). Charcoal rot caused by *Macrophomena phaseolina* on cantaloupe was significantly higher on plants infected with *R. reniformis* (Carter, 1980).

Economic importance

Tomato yield was reduced following inoculation with 100 nematodes/plant (Singh and Khera,

1979). Snake gourd (*Trichosanthus dioica*) plants inoculated with 1000 nematodes were stunted, had smaller leaves than the controls, and had brown roots with cortical necrosis (Nath *et al.*, 1979). The nematode affects a number of vegetable crops, which yield increases on okra, tomato, lettuce and squash of 19, 15, 57 and 69% obtained with granular nematicides, respectively (Heald, 1978).

Management

CULTURAL: a 2-year rotation of cotton with sorghum proved as effective as fumigation in reducing the nematode (Thames and Heald, 1974). Rotations that include soybean resistant to the nematode also reduce its densities (Gilman *et al.*, 1978). Nematode densities have also been reduced in rotations with maize, sugarcane and Pangola grass (Heald and Thames, 1982). A number of other crops are also known to reduce the nematode, including finger millet, groundnut, chillies, sugarcane and other grasses (Armstrong and Jensen, 1978; Bridge, 1983). In glasshouse experiments, groundnut was a poor host for two populations of *R. reniformis* (Germani, 1978).

Quénéhervé *et al.* (1998) suggested the use of *M. pruriens* over *T. erecta* and *Brachiaria decumbens* as a 3-month rotation crop prior to the cultivation of short-term vegetable crops such as lettuce. A 2-year rotation with *M. deeringiana* ploughed into the soil 3 months after planting versus non-incorporated was an effective control measure for reniform nematodes on tomato, with yield increases of 176% (Acosta *et al.*, 1995). Conversely, a number of winter cover crops incorporated into the soil before okra did not have a major effect on *R. reniformis* or on yield (Guertal *et al.*, 1998).

Populations of *R. reniformis* dropped 86% following soil solarization, and this was considered to provide season-long control on tomato, even under conditions of abundant rainfall and extended cloud cover (Chellemi *et al.*, 1993). Soil amendments, such as animal manure and cotton seed cake, have been used with success in controlling the nematode (Badra *et al.*, 1979).

BIOLOGICAL: very little has been undertaken in the area of biocontrol of this nematode, although

it would be a good target for antagonists. *P. lilacinum* reduced nematode densities and offset damage to tomato at midseason and at harvest in field and greenhouse microplots (Walters and Barker, 1994). Sitanshu *et al.* (2012) observed reduced nematode numbers and increased yield of bitter melon following application with *Pseudomonas fluorescens*, *P. lilacinum* or *P. chlamydosporia*. Sitaramaiah and Sikora (1982) demonstrated that the penetration and reproduction of *R. reniformis* on tomato and cucumber were reduced significantly in the presence of the endomycorrhizal fungus *Glomus fasciculatum*. The results indicated that crop rotations that facilitated mycorrhizae could be important in controlling *R. reniformis*.

RESISTANCE: there are only a few reports concerning resistance in vegetables to *R. reniformis*. In Egypt, the tomato cv. VFN 8 was shown to be moderately resistant to the reniform nematode (Oteifa and Osman, 1974). Balasubramanian and Ramakrishnan (1983) found that the tomato cultivars Kalyanpur Sel 1 and Sel 2 were immune to the reniform nematode, while lines EC 118272 and EC 118276 were resistant. Kumar (1995, 1998) reported resistance in one tomato cultivar and two aubergine lines.

CHEMICAL: a wide range of fumigant and non-fumigant nematicides are effective in controlling *R. reniformis* (Heald and Thames, 1982). The combination of nematicides and neem cake increased tomato yields and reduced reniform densities in field trials (Anitha and Subramanian, 1998). Rich and Bird (1973) were able to reduce nematode penetration by a single foliar application of oxamyl. However, McSorley (1980) could not demonstrate effective nematode control following six weekly sprays with oxamyl on snap bean. All granular nematicides tested in Martinique reduced *R. reniformis* densities in tomato plots (Cadet, 1990).

Soil solarization combined with carbofuran increased tomato yields by 96%, and solarization with neem cake reduced nematode densities and increased yields by 52% (Sharma *et al.*, 1996). In tests with cucumber, crop growth and yield when mulched with clear plastic for 5 weeks were significantly greater than under non-mulched conditions and were related to lower reniform nematode densities and not changes in soil fertility (Coates *et al.*, 1998).

Nacobbus

There are three recognized species of the genus, *N. aberrans*, *Nacobbus bolivianus* and *Nacobbus dorsalis* (Manzanilla-Lopez *et al.*, 2002). *N. aberrans* is the most commonly recorded species, although there appears to be a species complex (Reid *et al.*, 2003). One or more of these species are found in North, Central and South America. *N. aberrans* has been reported from cabbage, phaseolus beans, turnip, sweet pepper, chilli pepper, squash gourd, lettuce, tomato, cucumber and carrot.

Symptoms of damage

The nematode is often referred to as false root knot nematode and produces galls similar in size to *M. hapla*. The galls are characteristically produced in a bead-like fashion along the root. The penetration of juveniles and immature females into the root can cause root necrosis (Bridge, 1983). Stunting, poor growth and chlorosis are typical above-ground symptoms associated with this endoparasitic nematode. Yield reduction can be significant (Schuster *et al.*, 1965). *N. aberrans* is an important pathogen in Mexico, particularly on pepper, beans and tomato (Roman, 1978; Valesquez-Valle, 2001; Manzanilla-Lopez *et al.*, 2002).

The galls of *Nacobbus* spp. are often overlooked or mistaken for those produced by root knot nematodes, because of the similarity in gall form. Galls only occur in the presence of the adult females.

Vargas *et al.* (1996) demonstrated in greenhouse split-root tests that *N. aberrans* could break down plant resistance to *Phytophthora capsici* in *Capsicum annuum*.

Biology

The females, which vary greatly in shape, produce an egg sac that extends to the outside of the root (Clark, 1967; Johnson and Fassuliotis, 1984). According to Prasad and Webster (1967), the nematode completes a life cycle in 36 days at 25°C and in 43 days at 20 or 30°C. The genetic variability in *N. aberrans* and distinct geographical differences (Reid *et al.*, 2003) indicate that races may exist.

Management

Nacobbus can be controlled with both fumigant and non-fumigant nematicides. However, crop

rotation with non-host crops is effective and economical. Gomes (1973) reported that *Erodium cicutarium* and *B. campestris* were not susceptible, and hybrids of *Solanum andigenum* were resistant to the nematode. Bridge (1983) listed melon, squash, watermelon, groundnut, soybean, lucerne, oat, barley, rye, sorghum, wheat, maize, onion, okra, cotton, sunflower, *Phaseolus* spp., sesame, winged bean and rice as non-host crops that could be used in rotation. As races appear to exist, retesting each crop with local populations is suggested as a necessary precaution.

Intercropping tomato with *T. erecta*, independent of the planting date of *T. erecta* or spacing, showed a reduction in *N. aberrans* infection (Zavaleta and Gomez, 1995).

Resistance to *N. aberrans* has been identified and confirmed in a range of *Solanum* germplasm accessions, including those that possess genes for resistance to root knot nematodes (Veremis *et al.*, 1997). However, no resistant cultivars appear to be available yet. Lax *et al.* (2016) screened different pepper lines, but none were found to be resistant against *N. nacobbus*, not even those lines carrying *Me1* and *Me7* genes resistant to *Meloidogyne* spp.

Methods of diagnosis

The nematode is detected easily by examining the root system during the growing season. Attention should be paid to the size of the galls and their orientation along the root system. If they are small and form bead-like strands along the root, they should be examined for *Nacobbus* females, either by teasing out the females or by staining. However, when *Nacobbus* occurs in concomitant populations with *Meloidogyne*, it is often difficult to distinguish the different root galls.

Cyst Nematodes

Globodera

The potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida* will infect and damage tomato and aubergine. The potato cyst nematode infects tomato in North, Central and South America (Bridge, 1983). The nematode is also

present in Pakistan, India, the Mediterranean basin, South Africa, Kenya and the Philippines. Symptoms include chlorosis, stunting and general poor growth. At flowering, white, pinhead females are visible on the roots that later turn into brown cysts. Detailed studies on yield losses and control, however, have not been reported for either crop. Both species can be controlled easily by not planting solanaceous crops for 3–4 years and by controlling solanaceous weeds at the same time. Tomato cultivars harbouring the *Hero A* gene are highly resistant against all pathotypes of *G. rostochiensis* and *G. pallida* (Sobczak *et al.*, 2005).

Heterodera

The sugarbeet cyst nematode *H. schachtii* occurs in Mexico, the USA and Canada, Iraq, Libya, Senegal, Gambia, South Africa and Korea (Sikora and Fernández, 2005; Kwon *et al.*, 2016), causing significant losses on cruciferous crops. Yield reductions of 50% or more have been measured on Brussels sprouts, cabbage, broccoli and cauliflower when population densities are high (Miller, 1986; Zyl and Meyer, 2000). The nematode also attacks kale, Chinese cabbage, red beet, swede, spinach and turnip (Anon., 1987).

H. schachtii often occurs together with the cabbage cyst nematode, *Heterodera cruciferae*, and, since cysts on the root system of both nematode species are similar, proper identification is necessary in selecting control measures. Approximately 2–4 eggs/g of soil is used as the damage threshold in the Imperial Valley in California, USA (Anon., 1987). Winter-season crops and crops grown at higher altitudes are damaged less severely. The nematode is best controlled by rotation with non-host plants or resistant green manures, e.g. certain cultivars of mustard and fodder radish.

The cabbage cyst nematode *H. cruciferae* has been detected in California (Siddiqui *et al.*, 1973), Libya (Edongali and Dabaj, 1982) and Iran (Jabbari *et al.*, 2015). The nematode causes significant damage to cruciferous crops in California, where it often occurs together in the same fields with *H. schachtii* (Anon., 1987). Although the nematode has many hosts common to the sugarbeet cyst nematode, its host range is somewhat less (Johnson and Fassuliotis, 1984).

Seedlings infected with the nematode are stunted and exhibit interveinal chlorosis or leaf reddening (McCann, 1981). Cauliflower curd quality is reduced at 75 eggs/g of soil (Sykes and Winfield, 1966), and cabbage is severely stunted at 20 cysts/100 g of soil (McCann, 1981). Control is usually accomplished by crop rotation with non-host plants or pre-plant fumigation.

Cactodera

This cyst nematode attacks spinach in central Mexico (Sosa-Moss, 1986), was found on *Amaranthus viridis* in Cuba (Stoyanov, 1972) and was detected in Florida (G. Rau, unpublished, cited in Luc, 1986). The host range of the nematode is limited to *A. viridis*, *Amaranthus spinosus* and *Amaranthus retroflexus* (Luc, 1986). Golden and Raski (1977) discussed the biology of the nematode.

Methods of diagnosis

All these endoparasitic, sedentary nematodes produce cysts on the surface of the root system at specific times in their life cycle. The presence of cyst nematodes can be determined by removing growing plants carefully at different intervals during the growing season and examining the roots with a hand lens. The detection of cysts embedded in the root tissue is a clear sign of pathogenicity. Cysts can also be extracted from the soil using the techniques described in Chapter 4, this volume. The time of cyst appearance on the root surface is determined mainly by temperature. The cysts will also vary in colour, from white through beige to dark brown. Cyst production and detection will also vary depending on the number of life cycles produced; for example, the potato cyst nematode has just one generation per year, whereas the cabbage and sugarbeet cyst nematodes have many generations in a cropping season.

Ditylenchus

The genus *Ditylenchus* represents a species complex comprised of more than 80 recognized nematode species, of which just a few are pathogens of higher plants. One such pathogen is the

bulb and stem nematode *D. dipsaci*, causing severe damage on vegetables worldwide. A second species, *Ditylenchus destructor*, causes damage to carrot and garlic and is of minor importance. *D. dipsaci* has been reported attacking various semi-temperate vegetables and species of *Allium* in a number of subtropical and tropical countries: Mexico, Venezuela, Ecuador, Peru, Colombia and the Dominican Republic, and various countries in the Mediterranean, Asia and the Pacific (Bridge and Hunt, 1986; Pedroche *et al.*, 2013). In Morocco, the onion race of *D. dipsaci* (= *Ditylenchus gigas*) was reported as causing severe injury to garlic, onion and peas, with infection rates ranging from 55 to 100%. Overall, problems occur mostly during the winter season and in the cooler, upland tropical and subtropical regions (Pethybridge *et al.*, 2016). The nematode also causes severe injury to *Vicia faba* during the cool, rainy, winter growing season in the subtropical regions of North Africa (Saxena *et al.*, 1987). Vegetables growing in the warm tropics or during the summer season in the subtropics are not attacked by *D. dipsaci*.

Symptoms of damage

D. dipsaci is an endoparasite of over 500 angiosperms that infects aerial parts of plants, bulbs, tubers and rhizomes. Penetration of onion leaves causes leaf deformation and leaf swellings or blister-like areas on the surface. The leaves grow in a disorderly fashion and often hang as if wilted. As the season progresses, they become chlorotic (Decker, 1969; Marek *et al.*, 2005). Young plants can be killed at high levels of infections. Infected onions become swollen (bloat) and the bulbs may rot during storage (Bridge and Hunt, 1986). The inner scales of the bulb are usually attacked more severely than the outer scales. As the season advances, the bulbs become soft (Fig. 10.13) and, when cut open, show browning of the scales in concentric circles. Conversely, *D. dipsaci* on garlic does not induce deformation or swellings, but causes stunting and chlorosis of the above-ground plant part, underdevelopment and discoloration and splitting of bulbs, basal plate damage and reduced roots (Decker, 1969; Mollov *et al.*, 2012; Testen *et al.*, 2014). *D. dipsaci* on carrots causes swelling of the stem base (Fig. 10.14).



Fig. 10.13. Garlic infected by *Ditylenchus dipsaci*. (Photograph courtesy of J. Starr.)



Fig. 10.14. Thickening of stem base caused by *Ditylenchus dipsaci* on carrot. (Photograph courtesy of N. Greco.)

Biology

Fourth-stage juveniles penetrate the stem and leaf tissue through the stomata. Egg laying begins at temperatures of 1–5°C, with an optimum at 13–18°C. *D. dipsaci* completes one generation in 19–23 days at 15°C, depending on the host plant. Nematode activity stops at 36°C. The nematode prefers the cool, moist climatic conditions existing in the upland tropics and wet winter seasons in the subtropics. *D. dipsaci* can parasitize plants on both heavy and light soils, although a higher incidence seems to occur on heavy soils.

Races

D. dipsaci exists in numerous, at least ten, biological races (Sturhan, 1969). The oat and lucerne races are reported from eastern Australia (Perera *et al.*, 2009). The oat race breeds on

onion and has a polyphagous nature. However, information on the race spectrum in the tropics is lacking and the host range of many races has not been determined adequately. It should be noted that most crops are usually attacked simultaneously by populations containing a mixture of races, which often makes determination of threshold levels difficult. In Israel, two distinct races were identified, with one infecting onion and garlic but not *Phalaris canariensis*, whereas the second infected *Phalaris* and oat but not onion or garlic (Aftalion and Cohn, 1990).

Survival and means of dissemination

D. dipsaci can survive in the soil without a host plant for over a year, and the fourth juvenile stage can survive in anhydrobiosis for many years. Survival of the nematode basically depends on the type of population. As shown by Douda (2005), a chicory population of *D. dipsaci* survived on chicory but not on onion, garlic, leek or spinach, whereas a garlic population of *D. dipsaci* survived on onion and garlic, but not on leek, spinach or chicory. *D. dipsaci* can be disseminated by transportation in infected planting material, plant residue and adhering soil. Seed-borne infections are also responsible for long-distance dissemination in onion, broad bean, beet and lucerne. Other hosts and weeds are responsible for maintaining infections between onion and garlic. Bulbs harbouring mild infections will survive storage and increase the level of infection and losses in the following season when used as planting material. The nematode also attacks many weeds (Augustin and Sikora, 1989), which need to be examined for host status, since high nematode densities can be maintained on these hosts. Abbad and Bachikh (2001) detected the nematode in 11 of 60 weed species growing in *V. faba* fields in Morocco, including the parasitic plant *Orobanche crenata*. A number of weed hosts lacking symptoms of infection were detected in fields after garlic cultivation in Brazil (Fonseca *et al.*, 1999).

Economic threshold level

Knowledge on the damage threshold levels on economically important vegetable crops is essential for establishing appropriate control strategies. According to Seinhorst (1956), the economic

threshold level for *D. dipsaci* on onion is as low as 10 nematodes/400 ml of dry soil.

Management

Rotation with non-host crops for 3 years is the main method of control of *D. dipsaci*. However, the presence of morphologically indistinguishable host races with different host preferences generally makes rotation ineffective (Marek *et al.*, 2005; Dikici *et al.*, 2014). Early planting reduced *D. dipsaci* damage on onion (Mennan, 2005).

Fumigant nematicides reduce nematode infestation levels in the field but will not eradicate the nematode from the soil. The use of solarization combined with fumigation and granular nematicides increased the marketable yield of onion bulbs between 90 and 100% over the control (Lamberti *et al.*, 2001). However, solarization did not always improve results over individual chemical treatments, nor did the use of non-fumigant nematicides after fumigation. Good control was attained in Italy with solarization for 4 weeks in July or August, with 1,3-dichloropropene or metham sodium in the autumn, or fenamiphos just before transplanting in January or February (Lamberti *et al.*, 2000). Greco *et al.* (1992) reported that more onion plants survived *D. dipsaci* infection and greater yields were obtained with a combination of solarization and 1,3-dichloropropene. Non-fumigant nematicides were also effective in increasing marketable onion bulb yield and reducing soil nematode densities (Greco *et al.*, 1992; Jaehn and Kimoto, 1995; Sasanelli *et al.*, 1995). Zouhar *et al.* (2016) tested the nematicide potential of hydrogen cyanide and achieved up to 99% mortality of *D. dipsaci* when garlic was treated with HCN at a concentration of 20 g/m³ for 12 h in a fumigation chamber.

D. dipsaci can be controlled in onion bulbs by dipping in hot water at 44–45°C for 3 h (Bridge and Hunt, 1986). Temperature and time ratios are important for control and may vary with crop and cultivar. Jaehn (1995) reported that treatment of bulbs for 60 min in hot water at 49–50°C eradicated *D. dipsaci* from peeled garlic seed bulbs. Hot water treatment protocols depend mainly on the hot water tolerance of the crop. For instance, exposure for 30 min at 49°C slightly reduced emergence but did not affect the crop. Roberts and Matthew (1995) reported that abamectin at 10–20 ppm as a

20-min dip at 49°C or as a 20 min cool dip at 18°C following a 20 min hot water dip was highly effective in controlling *D. dipsaci* on garlic. Furthermore, sodium hypochlorite in a 1.052–1.313% aqueous solution as a 20-min hot dip was similarly effective. Both treatments were non-toxic to the plant. Immersion of infected garlic cloves in solutions containing abamectin, however, led to yields equal to that of the uninfected controls and were 56% higher than the untreated infected bulbs in trials conducted in California (Becker, 1999). In addition, 93% of the bulbs were nematode free compared with 46% in the nematode-infected control.

Methods of diagnosis

D. dipsaci can be detected easily by placing small pieces of plant tissue (seed, stem, leaf or bulb) into water. The mobile stages escape the tissues within 2–4 h, after which its presence can be checked by a microscope (EPPO, 2017). Rapid molecular methods are now available to distinguish *D. dipsaci* from other species within the genus (Subbotin *et al.*, 2005; Jeszke *et al.*, 2014). Detection in soil is more difficult because of the low population levels normally present. Consistent detection was obtained when sampling was conducted 1 week before harvesting by sampling plants adjacent to plants showing symptoms (Jaehn and Kimoto, 1994).

Pratylenchus and *Radopholus*

Root lesion nematodes (*Pratylenchus* spp.) and the burrowing nematode (*Radopholus similis*) are migratory endoparasites feeding among cortical root tissue. Their infection results in necrotic brown lesions. Water and nutrient uptake is affected, and wounds caused by the nematodes during penetration facilitate secondary pathogens, thereby increasing root damage. *Pratylenchus* is a major pathogen of most vegetable crops, causing severe yield and quality losses. Species associated with vegetables include *Pratylenchus brachyurus*, *Pratylenchus barkati*, *Pratylenchus dasi*, *Pratylenchus coffeae*, *Pratylenchus delattrei*, *Pratylenchus loosi*, *Pratylenchus singhi*, *Pratylenchus thornei*, *Pratylenchus zeae*, *Pratylenchus kumamotoensis*, *Pratylenchus pseudocoffea*, *Pratylenchus penetrans*, *Pratylenchus scribneri*, *Pratylenchus neglectus* and *Pratylenchus crenatus* (Hay and

Pethybridge, 2005; Waceke, 2007; Anwar *et al.*, 2013; Kim *et al.*, 2016). *P. brachyurus* and *P. zeae* have been especially detected in large numbers in vegetable roots. *P. penetrans* has been found associated with cucumber, tomato, chilli and bell peppers cultivated in plastic tunnels (Anwar *et al.*, 2013). This species also affects carrot yield and quality significantly, causing yield loss up to 40–80% (Teklu *et al.*, 2016) (Fig. 10.15). Damage caused by *Pratylenchus* spp. is further enhanced by co-infection with other nematode groups. For instance, Shakeel *et al.* (2013) reported higher damage on aubergine, okra, cucumber and tomato with simultaneous infection by *Pratylenchus* spp. and *M. incognita* than by either nematode alone. Unfortunately, the overriding importance of *Meloidogyne* in vegetable production has generally limited our knowledge as to the exact importance of lesion nematodes in vegetable production. *R. similis* has been detected in a number of vegetable crops, including kale, radish, tomato, aubergine, okra, carrot, onion, African spinach, watermelon, melon, calabash, pumpkin and squash (Anwar *et al.*, 2013). Crop loss studies have not been conducted.



Fig. 10.15. Intensive side root formation of *Pratylenchus penetrans* on carrots (left) compared with healthy carrot. (Photograph courtesy of J. Hallmann.)

Management

Lesion nematodes can be controlled with fumigant and non-fumigant nematicides, although this is probably not practical or economic. Many species of *Pratylenchus* have wide host ranges, including many weeds (Ntidi *et al.*, 2012), creating difficulties for rotations. A sequence of host and non-host crops could be combined with fallow (i.e. pea–French marigolds–fallow–carrot–bean–fallow), reducing *P. penetrans* populations by about 90% (Pudasaini *et al.*, 2006). A list of non-host plants for various species of *Pratylenchus* has been compiled by Armstrong and Jensen (1978). Inomoto *et al.* (2004) reported that *P. coffeae* failed to reproduce or damage okra under greenhouse conditions. Ornat *et al.* (1999) recommended short-term clean fallow combined with root destruction between successive crops in intensive vegetable production for control of *P. neglectus*. Direct incorporation or aqueous extracts from asparagus rootstocks effectively reduced *P. penetrans* up to 23% in lettuce, due to secondary metabolites (Uragami *et al.*, 2012). Essential oil extracts of *Ocimum gratissimum* and *Ocimum basilicum* resulted in the complete inhibition of *P. brachyurus* egg hatching and juvenile survival after 24 h exposure at 25–100 µg/ml in tomato. Minagawa *et al.* (2004) reported that a 4-week soil solarization could control *P. penetrans* effectively on carrots.

Methods of diagnosis

Lesion nematodes produce small, dark necrotic lesions on the root surface on many crops, which is the result of interrelationships with soil-borne fungal pathogens. The presence of lesions is a good indication that lesion nematodes are causing damage. The presence of the nematode should then be determined by extraction from the root tissue.

Belonolaimus

The sting nematodes, *Belonolaimus gracilis*, *B. longicaudatus*, *Belonolaimus euthorchilus*, *Belonolaimus maritimus* and *Belonolaimus nortoni* are common plant parasitic nematodes in the subtropical regions of the lower Coastal Plain of the south-eastern USA, from Virginia to Florida and

along the Gulf Coast into Texas. *B. longicaudatus* is reported causing yield loss of several vegetable crops, including carrot, chilli pepper, cabbage, cucurbits, aubergine, okra, pea, potato, celery, pumpkin, onion and tomato (Waceke, 2007; Anwar and McKenry, 2012). Physiological races of *B. longicaudatus* have been reported (Abu-Gharbieh and Perry, 1970).

Symptoms

Both males and females feed on cells of the root meristem, resulting in cell necrosis and cessation of root growth, which in turn induces stubby and abbreviated appearance of the roots (Han *et al.*, 2006). Damaged plants are stunted and chlorotic, and senesce prematurely, with severe damage levels leading to death. Perry and Rhoades (1982a) stated that 'infested areas consist of spots that vary in size and shape, but the boundary between diseased and healthy plants usually is fairly well defined'. Although disease complex associations have been detected on other hosts, they have not been reported from vegetable crops.

Biology

The nematodes are obligate parasites that cause damage to vegetables by feeding ectoparasitically on or near the root tip. The ectoparasite completes one generation within 28 days at an optimum temperature of 28–30°C, varying between populations. Temperatures above 33°C are generally detrimental to the nematode (Han *et al.*, 2006).

Survival and means of dissemination

There is no definite survival stage in the life cycle of the nematode, with all stages of development present in the rhizosphere. The nematode may have been spread to various warmer regions on infested Bermuda grass golf course turf (Perry and Rhoades, 1982a), but because of its dependency on extreme sandy soil (Thames, 1959; Brodie and Quattlebaum, 1970), establishment has probably only occurred in a limited number of instances. The nematode seems to be most damaging on irrigated light soils, due to the nematode's requirement for uniform soil moisture, sandy soil and temperatures of 25–30°C for survival and multiplication.

Other hosts

The nematode causes severe damage to most agricultural crops, including many wild plants and most vegetable crops (Graham and Holde- man, 1953; Good and Thornton, 1956; Robbins and Barker, 1973; Williams, 1974). Forage grasses, turf, cotton, soybean, strawberry, citrus and palm are also damaged by the nematode, whereas tobacco and watermelon are con- sidered non-hosts. Because of the presence of races, variation in host range between popula- tions should be expected.

Economic importance

B. longicaudatus is the only species of the genus that has been shown to cause serious crop loss to vegetables. The species was considered respon- sible for greater yield loss to vegetables in Florida than any other single plant pest of any type at the time (Perry and Rhoades, 1982a). The pres- ence of a single specimen in 100 ml soil is suffi- cient to cause severe damage to a vegetable crop.

Management

CULTURAL: the restrictions in the use of nemati- cides has stimulated interest in cultural prac- tices for controlling plant parasitic nematodes of vegetables. The addition of organic amend- ments that alter soil conditions has been shown to suppress the sting nematode because of its extreme sensitivity to changes in soil environ- mental conditions (Heald and Burton, 1968). The application of poinsettia, *Brassica* sp. and spurge shoot extracts resulted in about 99.5% of sting nematode control (Cox *et al.*, 2006). Ro- tations designed to reduce population densities are difficult because of the wide host range, lack of resistant cultivars and possible presence of races in the species. A number of non-hosts are listed by Armstrong and Jensen (1978). The nematode did not reproduce on *C. spectabilis* in glasshouse tests (Rhoades, 1964) and, in the field, a summer cover crop of hairy indigo prevented a population increase (Rhoades, 1976a; Rhoades and Forbes, 1986). Fallowing and summer cover crops also reduced popula- tions and increased yield (Rhoades, 1983). In field experiments, high populations developed on *T. patula*, whereas a low build-up was detected on joint vetch, *A. americana* (Rhoades, 1980).

CHEMICAL: nematicides were effective and have been widely used to control this nematode (Wil- liams, 1974; Perry and Rhoades, 1982a). Good control has been obtained with pre-plant fumi- gant and non-fumigant nematicide treatment of cabbage and onion (Rhoades, 1969), and with both granular and transplant water application of non-fumigant nematicides on cabbage (Rhoades, 1976b). Gilreath *et al.* (2005) ob- tained best results of in-bed application of chlo- ropicrin to control *Belonolaimus* spp., resulting in increased tomato yields. Populations of *Belo- nolaimus* on tomato decreased rapidly with the application of propylene oxide. In addition, the application of this chemical up to 760 l/ha in- creased the concentration of phosphorus and potassium, leading to higher tomato yield (San- tos and Gilreath, 2007). However, it should be noted that the most effective nematicides may have been withdrawn.

BIOLOGICAL: biological control is a very useful method for managing plant parasitic nematodes. High levels of *Pasteuria* sp. endospores were able to suppress *B. longicaudatus* populations (Luc *et al.*, 2010). The application of *B. firmus* was also effective for management (Crow, 2014).

Methods of diagnosis

The nematode is an ectoparasite and can be ex- tracted easily from sandy soils with modified Baermann dishes or sieving and elutriation techniques (see Chapter 4, this volume).

Trichodorus and *Paratrichodorus*

Species of stubby root nematodes, *Trichodorus* and *Paratrichodorus*, occur on vegetable crops throughout the world. Waceke (2007) detected both genera in 65% of the 22 cabbage farms in- vestigated in Kenya. *P. minor* is considered an im- portant limiting factor on vegetables grown in light soils in subtropical regions (Perry and Rhoades, 1982b). *P. minor* attacks a wide range of vegetable crops and most other cultivated crop plants (Rohde and Jenkins, 1957; Perry and Rhoades, 1982b). *Paratrichodorus allius*, *Par- atrichodorus mirzai* and *Trichodorus viruliferus* are considered important on sugarbeet, carrot and pepper, respectively. Species of the stubby root

nematodes generally prefer sandy or sandy-loam soils, but can be highly abundant in organic soils (Perry and Rhoades, 1982b).

Symptoms of damage

The nematodes feed ectoparasitically, mainly at the root tip, where damage suppresses root elongation, resulting in the stubby root appearance associated with these nematodes. The amount of damage to the root system varies with the vegetable crop, but is characterized by reduced size and fewer, shorter rootlets (Johnson and Fassuliotis, 1984). The roots become discoloured and necrotic as the season advances. Netscher (1970) reported that *P. minor* caused a 50% reduction in tomato root weight.

Plant growth is retarded and the foliage on stunted plants may become chlorotic and necrotic (Christie and Perry, 1951; Tomitaka *et al.*, 2011), while some vegetables wilt when exposed to moisture stress. The nematodes cause severe crop losses to a range of vegetable crops, including onion, tomato, pepper, aubergine, beet, broccoli, Brussels sprout, cabbage, cauliflower, Chinese cabbage, radish, swede, turnip, endive, lettuce, leek, potato and spinach (Hall *et al.*, 2007; Li *et al.*, 2010; Greco and Di Vito, 2011; Anwar and McKenry, 2012; Mwangi *et al.*, 2014; Malherbe and Marais, 2015).

Paratrichodorus spp. and *Trichodorus* spp. transmit Tobacco rattle virus (TRV) that causes corky ringspot disease of potato and is considered a major threat to potato yield and quality (Charlton *et al.*, 2010). *Paratrichodorus teres*, *Paratrichodorus pachydermus* and *Trichodorus similis*, for example, are the main vectors of TRV that infects several ornamental bulb crops in Europe (Zoon *et al.*, 2002). With globalization and the movement of tubers and bulbs from temperate countries to tropical/subtropical countries, TRV could increase in importance in the future. Although TRV has been reported from several vegetable crops, nothing is known about its damage potential on vegetables. The fact that TRV is transmitted at very low densities of the nematode makes its control challenging.

Management

The wide host range of the nematode makes its management by crop rotation difficult. Ploughing

reduced nematode numbers only moderately, whereas cropping black radish decreased densities greatly. Soil amendment with solid organic matter (grassy weed residues and lucerne pellets) reduced the population of *Paratrichodorus* sp. and enhanced tomato growth (Walker, 2007). *C. spectabilis* has been shown to be a non-host of the nematode, and when used as a cover crop will reduce nematode densities (Rhoades, 1964). *Asparagus officinalis* cv. *altilis* has also been shown to be a non-host, due to the production of a highly toxic glycoside (Rohde and Jenkins, 1958).

Fumigant and non-fumigant nematicides are effective in reducing initial damage and in giving the vegetable crop a head start on the nematode. However, nematode populations build up quickly (Perry, 1953). Shank-injected metam sodium was effective in controlling the disease in potato (Ingham *et al.*, 2007). In-furrow application of oxamyl to potato inhibited population increase of *P. allius*, due to its nematostatic action, but nematode mortality was low (Charlton *et al.*, 2010). Some of the carbamate and phosphate non-fumigant nematicides provide control for longer durations than the fumigants (Rhoades, 1969). Increased total marketable potato yield was obtained with the application of aldicarb, and fosthiazate ascribed to efficient control of *Paratrichodorus* spp. (Hafez and Sundararaj, 2006).

Flooding for 2 weeks followed by 2 weeks of drying reduced nematode populations significantly (Overman, 1964). The application of neem products as soil amendment on fallow land reduced *Paratrichodorus* over time. Populations of *P. minor* on tomato following solarization were similar to that achieved with methyl bromide and chloropicrin fumigation in Florida (Chellemi *et al.*, 1993). The application of brassica as green manures in combination with half the recommended rate of 1,3-dichloropropene reduced *P. allius* below economic threshold levels (Riga, 2011).

Longidorus, Paralongidorus and Xiphinema

These nematodes have been shown to be potential problems locally. They can cause severe damage, especially on sandy soils, and are probably often overlooked wherever root knot nematodes predominate.

Xiphinema ifacolum is distributed in several countries of Asia, Africa and South America. The nematode attacks several vegetable crops, including aubergine, tomato, pepper, onion, lettuce, carrot, broccoli, celery, cucumber, radish, potato and okra (Srivastava *et al.*, 2012; Pedroche *et al.*, 2013). The nematode reduced okra and pepper growth and yield in the field. *Xiphinema longicaudatum* severely depressed the growth of aubergine, even though it seemed to be a poor host for the nematode (Lamberti *et al.*, 1992). *Longidorus africanus* caused damage to lettuce in the subtropical regions of southern California and Pakistan. Patchy growth and wilted seedlings were observed together with leaf margin chlorosis (Radewald *et al.*, 1969). Nematode feeding caused a reduction in elongation of the taproot and root tip swelling, typical of damage by a number of species of *Longidorus* and *Xiphinema* on other crops. Carrot and lettuce seedlings were highly sensitive to early attack by *L. africanus* (Huang and Ploeg, 2001). Delaying damage in the seedling stage was considered important in reducing damage in the field. *Longidorus israelensis*, a parthenogenetic species, caused arrested root growth, root tip galling and deformed and forked tap roots. The nematode migrated to depths of 20–40 cm to survive hot, dry summer conditions (Peneva *et al.*, 1998). *Longidorus vineacola* was reported damaging celery in Israel (Cohn and Auscher, 1971).

Although viruliferous *Xiphinema americanum* have been found associated with watermelon, virus transmission does not appear to be a major problem in melon or vegetables in general (McGuire, 1982). The application of increasing amounts of sewage sludge to sandy soils led to a decrease in *Xiphinema basiri* and an increase in okra plant growth (Paulraj and Ramulu, 1994). Pelletized organo-mineral fertilizer applied as soil amendment at the rate of 10 Mt/ha reduced populations of *Longidorus* spp., resulting in increased fresh biomass of fluted pumpkin (Adekunle *et al.*, 2015). Yard waste compost, however, did not affect *Xiphinema* densities in Florida field trials (McSorley and Gallaher, 1995).

Other Nematodes of Vegetables

Stunt nematodes are often found associated with vegetables. Twenty-two species of *Tylenchorhynchus*

(three formerly named *Telotylenchus* and two *Quinisulcius*) and four species of *Merlinius* have been found in the rhizosphere of vegetable crops (Abd-Elgawad *et al.*, 2007; Chandra and Khan, 2011; Anwar and McKenry, 2012; Anwar *et al.*, 2013). With the exception of *T. brassicae*, none of the other species has been shown to be of significant economic importance on vegetable crops. *Tylenchorhynchus mashoodi* has been considered to be of potential importance on tomato.

T. brassicae has been detected in India, the Sultanate of Oman (Waller and Bridge, 1978) and Egypt (Oteifa and El-Sharkawi, 1965). The nematode is a serious problem for most cruciferous crops and, when high populations of this nematode occur, growth is affected negatively (Khan, 1969). Of 22 vegetables, cabbage and cauliflower were the most suitable hosts. Large differences in the response of cultivars to the stunt nematode exist. One cauliflower cultivar and two accessions were considered resistant to *T. brassicae* in greenhouse tests (Pasha and Tiyyagi, 1992). In combination with *R. solani*, the emergence of vegetable seedlings was reduced strongly (Khan and Saxena, 1969).

The awl nematode *Dolichodorus heterocephalus* can cause damage to vegetables, especially on wet, sandy soils. In Florida, the nematode causes severe damage to tomato and celery, with losses on heavily infested soil often exceeding 50% (Tarjan *et al.*, 1952; Perry, 1953; Johnson and Fassuliotis, 1984). The nematode causes stubby root symptoms and severe root necrosis, indicating an association with root-rotting fungi. The nematode can also attack the base of the hypocotyl, where necrotic tissues can be observed (Johnson and Fassuliotis, 1984).

Spiral nematodes *Helicotylenchus* spp. and *Scutellonema* spp. are commonly found in vegetable crops. Although more than 14 species of *Helicotylenchus* and three of *Scutellonema* have been detected in the rhizosphere of various vegetable crops, none has been shown to be of economic importance in the field.

Species of *Hoplolaimus*, *Aorolaimus* (syn. *Peltamigratus*) and *Zygotylenchus* have been found in soil samples from vegetable crops, but their importance has not been determined.

Six species of ring nematode, *Mesocrionema* (for correct genus terminology within criconematids see the Appendix in this volume), have been detected in the rhizosphere of a wide range

of vegetables. These nematodes are known to increase to high densities in many subtropical soils, and have been implicated as important limiting factors on a number of perennial crops and could be important on vegetables.

Future Prospects

Vegetable production is increasing in most subtropical and tropical countries in contrast to forced reductions in production being experienced in western Europe and North America. This is seen in the large increase in land under vegetable production in the tropics and subtropics since the 1990s (see [Tables 10.2](#) and [10.3](#)) – much of this relates to improved infrastructure, storage and transport systems and cheaper production. Similarly, this increase is associated with increased inputs in the form of fertilizers and pesticides, components that are being reduced in western Europe and North America, because of a reduction in farm subsidies and the public awareness of their impact on the environment. Emphasis must be placed on preventing the spread of new and important species to uninfested areas. This will be important as globalization expands and the movement of fresh produce increases. The need for taxonomists with molecular skills to identify the closely related species that occur simultaneously in the field has become evident.

Determination of threshold is necessary to aid the selection of specific control measures for pest management programmes. Whether precision farming technology can help in this direction is still unknown. Vegetables are often attacked simultaneously by a multitude of plant parasitic nematodes. This requires an expanded view of threshold levels, taking into account the effects of all the species involved. This is especially important where alternatives to fumigation are not available. Therefore, when determining damage intensity in the field, composite threshold levels, which include the interrelationships between all economically important nematode species, will be needed.

The loss of methyl bromide for root knot management has affected many commercial growers negatively around the world. At the

same time, it has simultaneously generated the impetus to establish effective alternatives – many of them discussed above in this chapter. It has also demonstrated the need to increase positions and research support in nematology. Alternatives are not identified just by wishful thinking alone or discussing farmer needs.

The development of resistant cultivars is playing an important role today and will increase in importance in the future, as will grafting on to nematode-resistant rootstocks. The development of transgenic cultivars is still not a reality, but could become an important tool for effective nematode management. The detection of resistance-breaking populations of *Meloidogyne* and new species able to break known genes for resistance underscore the need to stress resistance management.

If environmentally safe nematicides become available in the future, an increase in use can be expected in many subtropical and tropical vegetable-growing regions, if only because of the loss of methyl bromide and the toxicity of many non-fumigant nematicides that remain on the market. The use of more targeted delivery, such as seed coating, root drenches and monitored application through drip irrigation is relatively novel to nematology and will reduce many negative side effects of the past. A new generation of nematicides that are both effective and safe is needed urgently.

There will always be an imbalance in the availability of and access to pesticides between commercial and resource-limited growers, often due to cost implications. Besides, awareness is also a key aspect – of both the causal pest problems, as well as of the pesticides to treat them. This needs to change to make progress. In these regions, resistance and cropping systems research must be strengthened. Success with antagonistic plants, grafting, biofumigation, trap cropping and the management of antagonistic potential in the field must be explored further for integrated approaches that are economically acceptable.

Biological control is an alternative that has become a reality with the development of cost-effective, solid-state fermentation equipment and new formulations that reduce the costs of transport and facilitate application. Commercial production of biologically enhanced seed and transplants needs to be promoted for early root

protection. Suitable antagonists exist; the technology is available.

The 'all or nothing approach' to nematode control, or 'fumigate them', is outdated and in

the past. We need to improve 'nematode management' and maintain these pests at or below threshold levels. 'Living with them' is the concept of the future.

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11 Nematode Parasites of Groundnut*

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Groundnut is the common name for several leguminous plant species producing seeds that mature underground, including Bambara groundnut (*Vigna subterranea*), Hausa groundnut (*Macrotyloma geocarpum*) and peanut (*Arachis* spp.). Hausa groundnut is cultivated as a food crop primarily in West Africa and there are no reports of nematodes affecting this plant. Bambara groundnut is an important legume in sub-Saharan Africa, where it is grown primarily for human consumption (Mkandawire, 2007). Domesticated peanut (*Arachis hypogaea*) and Bambara groundnut will be the focus of this chapter.

Peanut is an annual, self-pollinating, herbaceous legume native to South America. It is referred to as groundnut in Africa and many parts of Asia. Peanut is a geotropic plant that produces its pods (fruit) underground. Flowering begins 4–6 weeks after planting and extends over a period of several weeks. Within about 1 week after the flowers are fertilized, pointed needle-like structures, carpophores, commonly called ‘pegs’, develop, elongate and grow into the soil to a depth of 2–7 cm. On entering the soil, the fertilized ovaries located behind the tip of the peg enlarge rapidly and pod growth begins. Two to four seeds are formed within a

pod. The length of time necessary for pod development to maturity may vary with cultivar and environmental conditions.

Peanuts are rich in calories and contain 25% protein. They may be boiled, broiled, roasted, fried, ground into peanut butter, or crushed for oil. This crop is grown primarily for cooking and salad oil, although peanuts are used in a variety of foods and snacks. Oil extraction also produces a protein-rich by-product that may be used for human consumption if processed from an edible-grade peanut; otherwise, it is used for animal feed.

Peanut is produced mostly in Asia (59.8%), Africa (29.8%) and the Americas (10.4%). Six countries – the People’s Republic of China (China), India, Nigeria, the USA, Sudan and Argentina – produce 74% of the world supply. Approximately 42.3 Mt of in-shell groundnuts are produced, with an average yield of 1.65 t/ha worldwide (FAOSTAT, 2014). Of the top producers, the USA (4.41 t/ha), China (3.49 t/ha) and Argentina (2.85 t/ha) have higher yields than the global average, while India, Nigeria and Sudan have lower. Production is distributed generally in tropical and subtropical regions of the world. Regions with loose, friable, sandy soils and warm temperatures are ideal for groundnut production.

*A revision of the chapter by D.W. Dickson and D. De Waele in the second edition.

Nematodes of Peanut

Plant nematodes are primary parasites of peanut in all production regions of the world. Based on a worldwide survey of nematologists, annual losses caused by all nematodes to peanut were estimated at 12%, and monetary losses were estimated at US\$1.03 billion (Sasser and Freckman, 1987). The primary nematode parasites include *Meloidogyne* spp., *Pratylenchus brachyurus*, *Belonolaimus longicaudatus*, *Aphelenchoides arachidis*, *Tylenchorhynchus brevilineatus* and *Ditylenchus africanus*. Each will be discussed in this chapter. Several other nematode species have been found associated with peanut (Sharma, 1985; Dickson and De Waele, 2005) but are not included herein.

Meloidogyne

Three major *Meloidogyne* spp. parasitize peanut and each is capable of causing severe suppression of peanut yields and fruit quality. *Meloidogyne arenaria* (peanut root knot nematode) and *Meloidogyne javanica* are highly virulent pathogens of peanut, whereas *Meloidogyne hapla* causes less damage but none the less is an important disease-inducing agent of peanut. All three species occur on peanut worldwide (Sasser, 1977). *M. arenaria* and *M. javanica* are common in warm and hot regions of the world, whereas *M. hapla* occurs in cooler regions. Two additional root knot nematode species parasitize peanut; however, their distribution is limited. In the USA, *Meloidogyne haplanaria* reduced peanut yields in Texas and has also been found in Florida and Arkansas (Eisenback *et al.*, 2003; Bendezu *et al.*, 2004; Joseph *et al.*, 2016). In China, *Meloidogyne panyuensis* was described from peanut roots in Guangdong Province (Liao *et al.*, 2005).

In the USA, *M. arenaria* is the dominant *Meloidogyne* species parasitizing peanut in Alabama, Florida, Georgia and Texas, while patchy occurrences have been reported in North Carolina, South Carolina and Virginia. In other regions of the world, *M. arenaria* is reported damaging peanut in Ghana (Osei *et al.*, 2005), Zimbabwe (Martin, 1958), Israel (Orion and Cohn, 1975), Egypt (Ibrahim and El-Saedy, 1976a), India (Dhurj and Vaishnav, 1981; Sakhuja and Sethi, 1985a), Taiwan (Cheng *et al.*, 1981) and China (Zhang, 1985).

M. javanica was first reported parasitizing peanut in Zimbabwe (Martin, 1958). Although the species is highly virulent on peanut, it is less frequently encountered on peanut in the USA than *M. arenaria*. It is only reported parasitizing peanut in Florida (Cetintas *et al.*, 2003), Georgia (Minton *et al.*, 1969b) and Texas (Tomaszewski *et al.*, 1994). The species is also known to occur on peanut in Egypt (Ibrahim and El-Saedy, 1976b), Brazil (Carneiro *et al.*, 2003) and India (Sakhuja and Sethi, 1985b; Sharma *et al.*, 1995).

M. hapla is encountered more frequently on peanut in northern latitudes. However, the nematode may be found at higher elevations in tropical regions (Eisenback and Triantaphyllou, 1991). *M. hapla* has been reported infecting peanut in all peanut-producing states in the USA except Florida (Dickson, 1998). It is frequently encountered infecting peanut in North Carolina, Virginia and Oklahoma. The species parasitizes peanut in Israel (Minz, 1956), South Africa (Van der Linde, 1956), Australia (Colbran, 1958; Saint-Smith *et al.*, 1972), Zimbabwe (Martin, 1961), Japan (Mitsui *et al.*, 1976), Korea (Choi, 1981) and China (Yang, 1984; Zhang, 1985).

Infection and histopathology

Second-stage infective juveniles (J2) of *Meloidogyne* spp. enter and damage peanut roots, pegs and pods. On entering root tips, they cause only slight mechanical injury, except when large numbers enter in a limited area. As a result of the juvenile feeding on vascular cells, multinucleate giant cells develop by the eighth day (Minton, 1963). Hyperplasia (increase in cell numbers) is observed in tissue adjacent to juveniles. Hyperplasia and hypertrophy (increase in cell size) result in the disorganization of vascular tissue and the formation of galled tissue. Elongation of severely galled roots is slowed, resulting in a stunted root system. A major consequence of root knot nematode development and giant cell formation is the malformation of the xylem elements and the inhibition of secondary growth of the xylem and phloem tissues. As a consequence, infected roots have impaired nutrient and water uptake.

Symptoms of damage

Noticeable above- and below-ground symptoms of root knot nematodes on peanut can be

observed as early as 45–75 days after planting, but most severe symptoms are observed after 90–120 days. Above-ground symptoms of root knot disease may be subtle or very conspicuous, especially as the crop nears maturity. The degree of symptoms depends on the growing environment as well as the juvenile density at the time of planting. In some cases, stunting of young plants may be severe. The general characteristics of diseased peanut plants are typical of other plants infected by these nematodes. As the crop nears maturity, heavily infected plants may be severely stunted, showing symptoms of chlorosis, incipient wilting, nutrient deficiencies, or even death when conditions are hot and dry (Fig. 11.1). Symptoms are distributed in patches of varying sizes. Infected plants exhibit a rusty, yellowish and mottled appearance. If drought occurs near the end of the season, the severity of root knot disease is accentuated and weakened plants die. Early season symptoms include stunted plants that fail to cover the soil between rows. The slowly dying and browning plants present a mottled effect among the greener plants, but these neighbouring plants, although they appear vigorous, are usually also

infected. In China, *M. arenaria*-infected plants may become yellow and stunted as early as 40 days after planting (Zhang, 1985).

Second-stage juveniles infect peanut plants soon after they germinate, but noticeable galling and egg masses are not apparent on the roots until 55–90 days after planting. The characteristic symptom on roots is the abnormal swelling (galls or knots) (Fig. 11.2); however, these are often difficult for the novice to see. Galled tissue on roots may attain a diameter larger than that of normal adjacent roots, but because of the abundance of nodules containing nitrogen-fixing bacteria (*Bradyrhizobium* sp.), the amount of galling is difficult to determine. This is distinct from what happens on tomato or cucumber, where galling is evident on roots 2–3 weeks after planting. Peanut root galls are small and generally discrete, compared with galls on other host crops, which can be large and become coalesced. Nitrogen-fixing nodules can be distinguished from galls by their distinctive round swellings attached to the root and are detached easily, whereas nematode galls are swellings that constitute a part of the fibrous root system and cannot be removed without destroying the integrity



Fig. 11.1. Peanut plants in the foreground (left) showing stunted growth and chlorosis from *Meloidogyne arenaria*; plants on the right are resistant to the nematode. (Photograph courtesy of T. Brennenman.)

of the root. Also, because root knot juveniles can infect nodules in some instances, galls may occur on the nodules, and vice versa. Juveniles may also infect pegs and pods after blooming and initiation of pod set (generally ~45 days after planting). Gallings on pegs and pods is distinctive and seen more easily than galling on roots, but interestingly, may not always occur, even though the roots are galled (Fig. 11.3). Situations where galling on pegs and pods is extensive generally result in a large reduction in potential yield. Yield potential is lost because pegs are weakened and easily fall off during harvest, pod formation is aborted, or damaged pods fail to produce seed. Gallings induced by *M. hapla*



Fig. 11.2. Peanut roots and developing pods with galls caused by *Meloidogyne arenaria*. (Photograph courtesy of P. Timper.)



Fig. 11.3. *Meloidogyne arenaria* infection of groundnut showing galls on pods and pegs. (Photograph courtesy of P. Timper.)

is distinctively different from that caused by *M. arenaria* or *M. javanica*. The former results in smaller galls, with some root proliferation above galls that results in a denser root system (Hirunsalle *et al.*, 1995a).

A key diagnostic element for root knot nematodes is the presence of female nematodes in galled roots, pegs or pods. Females are globose, approximately the size of a typewritten full stop (period) (800 μm length $\times$ 500 μm wide), pearly white in colour, and have sharp pointed necks and heads off to one side that are generally visible. An egg mass is generally extruded from the vulva end of each female at or near the root surface. This positions the egg mass on the outside surface of galled tissue, which facilitates both egg hatch and secondary infection of roots by the freshly hatched juveniles. Egg masses are about 1 mm in width, appear as brownish masses adhering to galled tissue and contain 300–500 eggs each. They are generally plentiful along galled tissue. Staining with food colouring (Thies *et al.*, 2002) or Phloxine B (Dickson and Struble, 1965) enables one to see them more readily.

M. hapla symptoms are generally less severe than those caused by *M. arenaria* or *M. javanica*. Above-ground symptoms of *M. hapla* may be difficult to detect because this pathogen causes less stunting or chlorosis. The most severe symptoms generally indicate large densities of infective juveniles in the soil. Severity over a field varies, depending on the differences in soil population densities and soil type. Sandy areas within a field often show the most severe symptoms. Plants with light infections usually are not obviously stunted or chlorotic. Typically, the only indication of root knot infection on such plants is galls on roots, pegs and pods.

Biological races

Among the root knot nematode species that infect peanut, there are two races reported for *M. arenaria* (Taylor and Sasser, 1978) and four proposed for *M. javanica* (Carneiro *et al.*, 2003). Of the two races of *M. arenaria*, race 1 infects peanut and race 2 does not; whereas, of the four proposed races of *M. javanica*, races 3 and 4 infect peanut, races 2 and 4 infect pepper and

race 1 infects neither. Host races of *M. arenaria* and *M. javanica* are morphologically indistinguishable (Sasser, 1979a; Carneiro *et al.*, 1998, 2003), thus their separation depends on their reaction on differential host plants. Most *M. javanica* populations do not reproduce on peanut (Taylor and Sasser, 1978). The worldwide distribution of populations of *M. javanica* that infect peanut is listed above.

Survival and means of dissemination

Plant nematodes are moved by humans, animals, water, wind and any other means that move soil or infected parts of plants. Humans and animals that track across fields infested with plant nematodes may potentially spread nematodes to uninfested fields via soil adhering to their feet. Important among dispersal methods is the movement of soil from infested fields on farming implements. Any type of cultivation equipment with soil adhering will move nematodes, but peanut producers must look to their diggers and combines as a principal means of moving root knot nematodes. Plant nematodes may be dispersed via the movement of freshly dug pegs, pods or roots; however, the developmental stages of nematodes generally do not survive in these plant parts when they are well dried. Interestingly, wind and water play a major role in the dispersal of nematodes. Second-stage juveniles of *Meloidogyne* spp. were among 28 genera recovered from dust traps placed 2 m above the ground in western Texas, USA (Orr and Newton, 1971). Dispersal by surface runoff water and by irrigation also occurs (Meagher, 1967; Sauer, 1968). Refuse from packing and processing plants that has not been thoroughly dried may harbour viable eggs and infective juveniles.

Environmental factors affecting parasitism

Temperature is considered particularly important for *Meloidogyne* spp. survival and parasitism, and the lower and upper temperatures for survival are approximately 0–5°C and 35–40°C, respectively (Taylor and Sasser, 1978). In general, the optimum temperature for the survival of eggs and juveniles is 10–15°C (Bergeson, 1959; Thomason *et al.*, 1964). The optimum temperature for hatching of *M. hapla* and *M. javanica* is 25 and 30°C, respectively (Bird and Wallace,

1965). *M. javanica* had a significantly higher hatch rate at 30°C than *M. hapla*. At 14.3°C, the life cycle of *M. javanica* requires 56 days, but only 21 days at 26.1°C (Milne and Du Plessis, 1964).

There is general agreement that *Meloidogyne* spp. damage is greater in sandy soils compared with heavier clay soils. In China, the incidence and severity of *M. arenaria* on peanut were related to soil texture (Zhang, 1985).

Soil moisture is necessary to sustain the activities of *Meloidogyne* spp. In moist soils of 40–60% field capacity, juveniles are active and move through the soil in a film of water surrounding soil particles. In dry soils, they become inactive and die through desiccation (Van Gundy, 1985). In wet soils, hatching may be inhibited and juvenile movement slowed by lack of oxygen. All activity of *M. javanica* increased as the oxygen concentrations increased from 0.2 to 21%, and it was concluded that a favourable environment would be provided when moist soils drained rapidly and allowed oxygen concentrations to increase above 10% (Baxter and Blake, 1969). *M. arenaria* was less damaging in fields that had a high water table than in well-drained fields (Zhang, 1985). Also, *M. arenaria* is less damaging to a peanut crop that follows a flooded crop than in fields that have not been flooded.

Disease complexes

Sclerotium rolfsii (southern blight) and *Cylindrocladium parasiticum* (Cylindrocladium black rot (CBR)) are two important soil-borne diseases that are associated quite frequently with root knot nematodes in peanut fields (Melouk and Backman, 1995). Because the incidence of southern blight, which causes peanut stem rot (Brenneman *et al.*, 1995), is reduced when crop rotation or nematicides are used to manage *M. arenaria* infection, the nematode is suspected of interacting with the fungus in a disease complex (Rodríguez-Kábana *et al.*, 1982a, 1994). However, a microplot study failed to show an interaction between *M. arenaria* and *S. rolfsii* in peanut (Starr *et al.*, 1996). Additionally, the suppression of *M. arenaria* with aldicarb in several studies has either found no change in the incidence of southern blight or, alternatively, an increase (Minton *et al.*, 1991; Rodríguez-Kábana *et al.*, 1994; Timper *et al.*, 2001). The evidence for a disease complex between root knot nematodes

and *C. parasiticum* is stronger than with *S. rolsfii*. Significant positive correlations between final densities of microsclerotia of *C. parasiticum* and *M. hapla* in a field test indicated that this nematode could affect CBR development (Diomandé and Beute, 1981). It was also found that *M. arenaria* and *M. hapla* increased the severity of CBR on both CBR-resistant and -susceptible peanut (Diomandé *et al.*, 1981; Culbreath *et al.*, 1992). In another study, *C. parasiticum* in the presence of *M. arenaria* increased the root rot ratings and plant mortality in two nematode-susceptible genotypes compared to the fungus alone, but had no effect on disease or plant mortality with a *Meloidogyne*-resistant genotype (Dong *et al.*, 2009).

A synergistic interaction in peanut pod rot and damping off occurred when *Pythium myriotylum* was combined with *Fusarium solani* and *M. arenaria* (Garcia and Mitchell, 1975a,b). Peanut plants inoculated with both *F. solani* mycelium and *M. arenaria* wilted sooner after inoculation than when *F. solani* was inoculated alone (Patel *et al.*, 1985).

Aflatoxins are potent carcinogens produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. In glasshouse and microplot studies, neither *M. arenaria* nor *M. hapla* increased aflatoxin contamination in peanut seed, even though the incidence of *A. flavus* increased in the seed with *M. hapla* (Minton and Jackson, 1967; Minton *et al.*, 1969a). These early studies were conducted before it was understood that aflatoxin production was promoted by drought stress and high temperatures. In two subsequent studies conducted under drought conditions, *M. arenaria* increased aflatoxin concentrations in peanut seed when levels of *A. flavus* and *A. parasiticus* were low, but the nematode had no effect on aflatoxin concentrations when levels of these fungi were high (Timper *et al.*, 2004, 2013). Galling of peanut pods may result in greater invasion of the pods by toxigenic *Aspergillus* spp. However, aflatoxin concentrations were also greater in peanut seed when *M. arenaria* infection was restricted to the roots, indicating physiological changes that increased plant susceptibility to aflatoxin contamination (Timper *et al.*, 2013).

The interaction of concomitant populations of *M. arenaria* races 1 and 2, and *M. hapla* on peanut showed that *M. arenaria* race 2 tended

to depress *M. arenaria* race 1 development, whereas *M. hapla* had little effect on the latter (Hirunsalle *et al.*, 1995a). Although the reproductive potentials of *M. arenaria* and *M. hapla* are similar, interaction studies between them on five peanut genotypes show that *M. arenaria* has greater infection capacity and causes greater damage than *M. hapla* (Hirunsalle *et al.*, 1995b). Thus, *M. arenaria* is more competitive than *M. hapla* on peanut. Infection and reproductive potentials and crop damage, induced by mixed populations, were similar to those induced by *M. arenaria* alone.

Economic importance and population damage threshold levels

Yield suppression by plant nematodes is difficult to estimate because damage is seldom confined to a single nematode species. Also, damage caused by low to moderate densities of plant nematodes often goes unnoticed. Where damaging levels of *M. arenaria* or *M. javanica* occur, more than 50% of yield potential can be lost. Even 100% losses have been observed in sections of severely infested fields; however, because of the uneven distribution of plant nematodes, losses over large fields may average less than 50%. In three nematicide efficacy trials conducted in peanut fields heavily infested with *M. arenaria*, the most effective nematicide treatments increased yields by an average of 83% (Dickson and Hewlett, 1988). Statewide annual yield losses due to *M. arenaria* generally are less, ranging from 0.5% in Oklahoma to 5% in Alabama, and from 0.3% in Georgia to 5% in North Carolina for *M. hapla* (Anon., 1987). Suppression of yields by *Meloidogyne* spp. in West Africa and South-east Asia were estimated at 15% (Sasser, 1979b).

In the southern USA, up to 40% of peanut fields were estimated to be infested by *Meloidogyne* spp. (Ingram and Rodríguez-Kábana, 1980; Sturgeon, 1986; Wheeler and Starr, 1987). In the Punjab, India, *Meloidogyne* spp. juveniles were present in an average of 47% of soil samples and galling observed in 31% of sites from three peanut-growing districts (Sakhujia and Sethi, 1985c). In Egypt, 65% of soil and root samples collected from fields with poorly growing peanut contained *Meloidogyne* spp., mostly *M. javanica* but also a few *M. arenaria* (Ibrahim

and El-Saedy, 1976a). In Guyana, 75% of soil samples collected around peanut plants contained *Meloidogyne* spp. (Singh, 1972). A large percentage, up to 61%, of peanut grown on 6200 ha in Leizhou Peninsula, China, was infected with *M. arenaria* (Zhang, 1985).

Several scientists have reported on the economic damage level of plant nematodes on peanut. Advisories for damaging levels of plant nematodes are usually based on juvenile density in the soil, because most extraction procedures do not recover nematode eggs from soil (Rodríguez-Kábana *et al.*, 1986). However, the timing of sampling is critical, because soil densities of *M. arenaria* juveniles at planting time are usually relatively low or near undetectable levels. Hence, for grower advisory purposes, it is usually best to determine population densities as soon after harvest of the previous crop as practical. Once root knot disease is observed on peanut, the problem will continue to increase unless the nematode is suppressed by natural antagonists or other causes (Dickson *et al.*, 1994).

In India, peanut plants were stunted by *M. javanica* when inoculated with 1 egg/cm³ soil (Sakhuja and Sethi, 1985b). A reduction of 27% in shoot length and 55% in shoot dry weight was obtained when plants were inoculated with 8 eggs/cm³ soil. In nematicide experiments, yields are usually correlated negatively with the density of *Meloidogyne* juveniles in soil. Regression analysis on data from 16 peanut experiments in Alabama indicated that yields were negatively related to *M. arenaria* juvenile density in the soil determined near harvest (Rodríguez-Kábana *et al.*, 1982b). On the basis of a linear regression model, it was determined that peanut yield loss in microplots was 8.6% for each tenfold increase in initial soil density of *M. hapla* juveniles (Rickard *et al.*, 1977). A significant negative relationship between initial population densities of *M. arenaria* in microplot tests and peanut yields was reported (Wheeler and Starr, 1987). A linear model estimated a 10% yield loss with initial densities of 44–83 eggs and juveniles/500 cm³ soil. In Florida, the damage threshold was estimated to be as low as 1 juvenile/100 cm³ soil (McSorley *et al.*, 1992). An inoculum density of 1000 *M. arenaria* juveniles/kg soil caused a reduction of peanut plant shoot growth, shoot weight and root length of 23.9, 33.1 and 31.9%, respectively (Dhurj and Vaishnav, 1981).

Management

Where root knot disease of peanut exists, some means of management is generally required for profitable peanut production. Each field should be evaluated based on the history of nematode damage before determining which management tactics to employ. The first line of defence should be to prevent further development of the disease by reducing spread. Management of root knot nematodes is very difficult and costly once it becomes established, in terms of both time devoted to developing management tactics and resources that must be allocated.

CROP ROTATION: one of the most effective means of management is crop rotation, using plants that are non-hosts or resistant to *M. arenaria*, *M. hapla* or *M. javanica*. When the cash value for peanut is low, this may be the only management tactic that can be used profitably. The objective is for peanut to follow poor or non-hosts for root knot nematodes, such as cotton, maize, small grains and pasture grasses (Bailey, 1988; Hagan, 1988; Dickson and Melouk, 1995; Dunn and Dickson, 1995); however, it is important to realize that the use of rotation varies with the nematode species present, the cultivar of rotational crop and the economics of growing a rotation crop.

Growing tropical forages for several years, e.g. bahia grass (*Paspalum notatum*), has long been recognized as one of the best rotations to precede peanut (Norton *et al.*, 1977; Rodríguez-Kábana *et al.*, 1994). Rotations of 3 or more years out of peanut and other favourable hosts are preferable to 1- or 2-year rotations (Dickson and Hewlett, 1989); however, for such a rotation to work successfully, weeds in the forage grasses must be managed properly. A few common weeds in the USA that occur frequently in the planting of forage grasses include hairy indigo (*Indigofera hirsuta*), alyce clover (*Alysicarpus vaginalis*) and morning glory (*Ipomoea* spp.), each of which is a good host for *M. arenaria*. Another tropical forage, coastal Bermuda grass (*Cynodon dactylon*), failed to increase peanut yields or decrease densities of *M. arenaria* on peanut after a 3-year rotation (Rodríguez-Kábana *et al.*, 1994). Also, Bermuda grass failed to reduce the incidence of southern blight (*S. rolfsii*), whereas both bahia grass and cotton did.

Cotton (*Gossypium hirsutum*) is a good rotation crop with peanut in situations where a single producer grows these two crops. *M. arenaria*, *M. hapla* and *M. javanica* do not reproduce on cotton, whereas *Meloidogyne incognita* races 3 and 4 that infect cotton do not infect peanut (Sasser and Carter, 1982; Rodríguez-Kábana *et al.*, 1994). Peanut yields were increased and *M. arenaria* densities reduced following 1 year of cotton (Rodríguez-Kábana *et al.*, 1987), although longer rotations were more effective in reducing nematode densities and increasing yields (Jordan *et al.*, 2008).

The susceptibility of various crops to *M. arenaria*, *M. hapla* and *M. javanica* is available from the literature. Seven of 30 crop plants tested in Taiwan were resistant (non-host) to *M. arenaria* (Cheng *et al.*, 1981). Although plants are listed as resistant or non-hosts, care must be exercised in the selection of cultivars, as there is often great variability within a crop species in the way they respond. A good example is maize (*Zea mays* L.), which, although reported as an excellent rotational crop with peanut, some cultivars can support relatively high population densities of *M. arenaria* and *M. javanica* (Baldwin and Barker, 1970; Norse, 1972; Windham and Williams, 1994). Conversely, maize is resistant to *M. hapla* (Baldwin and Barker, 1970), thus it is a suggested rotational crop for managing *M. hapla* on peanut in Queensland, Australia (Broadley, 1981; Vance, 1981). Yet growing maize in a peanut rotation is better than continuous peanut or other good hosts of root knot nematodes, such as soybean, tobacco or vegetables, all of which are excellent hosts of root knot nematodes, except when root knot nematode-resistant soybean or tobacco is planted. Grain sorghum (*Sorghum vulgare*) appears to suppress root knot nematode densities several-fold better than maize (Rodríguez-Kábana and Touchton, 1984; McSorley and Gallaher, 1991). Some grain hybrids of pearl millet (*Pennisetum glaucum*) are resistance to *M. arenaria* and *M. javanica* (Timper *et al.*, 2002; Timper and Hanna, 2005). In a field study, rotating peanut with 2 years of a nematode-resistant pearl millet was similar to maize in reducing root galling and improving yields of peanut compared to 2 years of a nematode-susceptible pearl millet (Timper *et al.*, 2007).

Some unusual crops, such as partridge pea (*Cassia fasciculata*) and American joint vetch

(*Aeschynomene americana*) suppressed soil densities of *M. arenaria* when either crop was grown for 2 years in rotation with peanut (Rodríguez-Kábana *et al.*, 1991). Partridge pea reduced *M. arenaria* more than joint vetch, but peanut yields were increased after 2 years of joint vetch. Sesame (*Sesamum indicum*), castor (*Ricinus communis*), hairy indigo (*I. hirsuta*) and bahia grass (*P. notatum*) were also promising for managing *M. arenaria* in peanut (Rodríguez-Kábana and Morgan-Jones, 1987). No *M. arenaria* galling was observed on American joint vetch, castor, cotton, crotalaria (*Crotalaria spectabilis*), sorghum–Sudan grass (*Sorghum bicolor* × *Sorghum sudanense*) or resistant soybean (*Glycine max*) when grown in microplots, whereas hairy indigo supported only a low level of galling (McSorley *et al.*, 1994).

The use of flood fallowing in conjunction with crop rotations may reduce root knot nematode damage effectively (Zhang, 1985), although in most instances this is not practical. Rotating a winter small-grain crop with peanut can help prevent the growth of weeds that are hosts of peanut nematodes. However, since some small grain cultivars may also support low population densities if grown during warm weather, planting should be delayed until cool weather, when nematode development and reproduction are reduced (Dunn and Dickson, 1995).

Crop rotation should not be expected to reduce a root knot nematode population abruptly, because: (i) some nematodes will survive the winter without a host; (ii) most crops can support at least some nematode reproduction; and (iii) most fields have some weeds that support nematode reproduction. Rotation is a far better tool to help keep relatively low densities from becoming too high, or for gradually reducing high densities over several years (Dunn and Dickson, 1995).

OTHER CULTURAL METHODS: destruction of the roots of host crops that precede peanut in a rotation will interrupt reproduction and help to reduce potential damage. Ploughing soils several weeks before applying nematicides and planting peanut encourages the decay of live plant roots that protect nematodes from their enemies or from nematicides that are applied to the soil (Dunn and Dickson, 1995). Drying soils after they have been turned may reduce plant nematodes (Zhang, 1985). Clean fallowing for

prolonged periods may also be effective. Tillage can have a small, but measurable effect on root knot nematodes. Compared to conventional tillage (mouldboard plough), reduced tillage (strip tillage) had 11–12% greater root galling (Minton *et al.*, 1990; Minton *et al.*, 1991; Timper *et al.*, 2011). Conventional tillage may disperse juveniles from the root zone or expose eggs and juveniles to desiccation and high temperatures near the soil surface.

In China, growers who apply ample fertilizers, especially organic fertilizers, have fewer problems with root knot nematodes than growers who use less fertilizer (Zhang, 1985). *M. arenaria* was less damaging in soils with high water tables than in well-drained soils; it was also less serious in irrigated than in non-irrigated fields (Zhang, 1985).

RESISTANCE: the most exciting development for nematode management is the availability of peanut cultivars with root knot nematode resistance. A systematic search was made of the *A. hypogaea* germplasm collection for useful sources of resistance (Holbrook and Noe, 1992). Several lines had moderate levels of resistance, although none had high levels of resistance. Resistance identified in *A. hypogaea* suppressed population densities of *M. arenaria* by 40–60% (Noe *et al.*, 1992). Resistance to *M. hapla* (Castillo *et al.*, 1973; Subrahmanyam *et al.*, 1983) and to *M. javanica* (Sakhujia and Sethi, 1985c; Ravindra *et al.*, 2013) was also reported in *A. hypogaea*.

A major breakthrough occurred when resistance to *M. arenaria* was found in 21 *Arachis* spp. and two interspecific hybrids (Nelson *et al.*, 1989). They also reported resistance to *M. hapla* in two *Arachis* spp. and one of the interspecific hybrids. There is research in the USA and Brazil focusing on the introgression of resistance to root knot nematodes from wild *Arachis* spp. into cultivated peanut. As *A. hypogaea* is an allotetraploid, and most wild species are diploids, the introgression of nematode resistance genes from the wild species into cultivated peanut is a difficult process. The introgression of resistance genes has followed three pathways. One pathway created a complex hybrid (TxAG-6) of three wild species that was cross-compatible with *A. hypogaea* (Simpson *et al.*, 1993). All three wild species, *Arachis batizocoi*, *Arachis cardenasii* and *Arachis diogeni* are resistant to *M. arenaria*, while

A. cardenasii is also resistant to *M. hapla* (Nelson *et al.*, 1989). Multiple genes for resistance to root knot nematodes were introgressed into *A. hypogaea* from TxAG-6 (Church *et al.*, 2005; Burow *et al.*, 2014); however, many of these genes were not carried forward in the back-crossing programme to develop peanut cultivars. The main source of resistance in cultivars containing genetic material introgressed from TxAG-6 was derived from *A. cardenasii*, and was dominantly inherited (Burow *et al.*, 1996; Choi *et al.*, 1999). This resistance is expressed as nearly complete inhibition of development by invading juveniles. The second pathway introgressed resistance to *M. arenaria* using *A. cardenasii* (Stalker *et al.*, 1994). High levels of resistance were introgressed into *A. hypogaea* using colchicine to create a hexaploid in an *A. hypogaea* × *A. cardenasii* hybrid, self-pollinating the hybrid and then selecting progeny that had reverted to a tetraploid. This effort led to the release of two advanced germplasm lines, GP-NC WS5 and GP-NC WS6 (Stalker *et al.*, 2002). Resistance in these germplasm lines is conditioned by two closely linked dominant genes: one reduces egg production and the other reduces gall formation (Garcia *et al.*, 1996). The gene conditioning resistance to egg production may be the same as the major effect gene(s) introgressed from TxAG-6 (Burow *et al.*, 2014). The third introgression pathway was initiated by creating a diploid hybrid between *A. batizocoi* and *Arachis stenosperma*, followed by chromosome doubling with colchicine to create an allotetraploid that was sexually compatible with *A. hypogaea* (Leal-Bertioli *et al.*, 2015). *A. stenosperma* is highly resistant to *M. hapla*, *M. arenaria* and *M. javanica* race 4 (Leal-Bertioli *et al.*, 2016). There appears to be two mechanisms of resistance operating in this species: fewer juveniles penetrate the roots of *A. stenosperma* than susceptible plants, and the juveniles that penetrate are prevented from developing by a rapid hypersensitive response in the cells surrounding the nematode feeding site (Proite *et al.*, 2008; Guimarães *et al.*, 2010, 2015; Morgante *et al.*, 2013).

Both random amplified polymorphic DNA (RAPD; Burow *et al.*, 1996; Garcia *et al.*, 1996) and restriction fragment length polymorphism (RFLP; Choi *et al.*, 1999) markers linked to the resistance locus in breeding lines derived from

A. cardenasii have been identified. However, these markers were not suitable for marker-assisted selection in breeding programmes. More recently, a dominant sequence characterized amplified region (SCAR) marker and a co-dominant simple sequence repeat (SSR) marker have been developed, both of which amplify fragments of different molecular weight from resistant and susceptible plants, thus reducing the risk of false negatives caused by failed reactions (Chu *et al.*, 2007, 2011). Interestingly, one of the SCAR markers for resistance transferred from TxAG-6 is not present in GP-NC WS6, indicating that different resistance genes may have been transferred from *A. cardenassi* using the two introgression pathways (Dong *et al.*, 2008). Kompetitive Allele Specific PCR (KASP) markers have also been developed for three loci in *A. stenosperma* associated with reduced galling and egg production by *M. arenaria* (Leal-Bertioli *et al.*, 2016). The location of these markers in the peanut genome is different from the location of the markers for the resistance derived from *A. cardenassi*, indicating that they are different genes. These markers will greatly expedite the transfer of resistance genes into peanut cultivars.

The first resistant peanut cultivars, COAN and NemaTAM, were developed from TxAG-6, which was backcrossed with cv. Florunner as the recurrent parent (Simpson and Starr, 2001; Simpson *et al.*, 2003). Both resistant cultivars suppress nematode reproduction by more than 90%, and have significantly greater yield potential than susceptible cultivars in fields where nematode population densities are high. However, as a result of low yield potential in the absence of nematodes, cv. COAN is no longer available (Starr *et al.*, 2002). The cv. NemaTAM has not been widely planted in south-eastern USA because of its susceptibility to Tomato spotted wilt virus (TSWV), a disease that regularly causes severe yield losses in that region. Subsequently, two other cultivars (Tifguard and Georgia-14N) were developed by crossing COAN with TSWV-resistant cultivars; both cultivars have a high level of resistance to root knot nematodes and TSWV, and have good yield potential in the absence of nematodes (Holbrook *et al.*, 2008; Branch and Brennenman, 2015). The cultivar Webb, which was developed from a cross between the cultivars NemaTAM and Tamrun OLO2, had a high resistance to root knot nematodes

and a moderate resistance to Sclerotinia blight caused by *Sclerotinia minor* (Simpson *et al.*, 2013). The high oleic/low linoleic fatty acid trait is important to the peanut industry because it extends the shelf life of peanut products; therefore, this trait has been integrated into several of the nematode-resistant cultivars (Webb, Georgia 14-N and TifNV-HighO/L).

To date, all cultivars resistant to root knot nematodes contain an alien chromosome segment derived via TxAG-6 from *A. cardenassi*. This segment is on chromosome A09 and is inherited as a single dominant gene. Although recombination in this segment of alien chromosome is greatly reduced (Nagy *et al.*, 2010), recombination within this segment has recently been observed (Branch *et al.*, 2014; Chu *et al.*, 2016). There is now evidence of two genes for resistance to root knot nematodes within this alien segment (Chu *et al.*, 2016). In recombinant lines containing different segments of the alien introgression, one small region confers a high level of resistance and a larger region confers moderate resistance. Although resistance to root knot nematodes is expressed as a hypersensitive response in *A. cardenassi* (Nelson *et al.*, 1990), a hypersensitive response was not consistently observed in advanced breeding lines containing the alien chromosome segment from this wild species (Choi *et al.*, 1999). Instead, juveniles readily penetrate roots of these resistant lines, but for some unknown reason fail to develop. In addition to resistance to *M. arenaria*, COAN, NemaTAM and C724-25-8 (a related breeding line of Tifguard) also have a high level of resistance to *M. javanica*, whereas GP-NC WS6 has only a moderate level of resistance to *M. javanica* (Dong *et al.*, 2008). Data from earlier breeding lines derived from TxAG-6 indicate that genes conditioning resistance to *M. javanica* differ from those that condition resistance to *M. arenaria* (Abdel-Momen *et al.*, 1998). As of 2017, no cultivar has a high level of resistance to *M. hapla*, although COAN and GP-NC WS6 have moderate levels of resistance to this species.

Shifts in root knot nematode species predominance due to the introduction of specific resistance genes have been documented for tobacco (Fortnum *et al.*, 1984). The availability of multiple genes for resistance to the major species of root knot nematodes infecting peanut is likely to be an important asset, in that it will allow the

development of gene deployment systems to enhance the durability of resistance currently being developed.

CHEMICAL: nematicides are one of the most reliable and efficient methods of managing important nematode diseases of peanut. In cases of severely infested fields, it may be the only effective choice, especially where crop rotation or resistant cultivars cannot be employed. There are two general types of nematicides available, fumigants and non-fumigants. The former is formulated as a liquid that, when applied into the soil profile, volatilizes to form a gas that is distributed uniformly through soil pore spaces, contacting and killing nematodes. The only two fumigant nematicides remaining on the market are 1,3-dichloropropene (1,3-D) and metam sodium. Both fumigants must be applied about 7 days (1,3-D) or 14–21 days (metam sodium) pre-plant to avoid possible phytotoxicity, and may be applied as a plough-down treatment as soil is prepared for planting, or by chisel injection. The fumigants are highly volatile; thus, it is essential that they be applied and sealed properly (Rodríguez-Kábana and Robertson, 1987; Riegel *et al.*, 2000a,b). Relatively high rates (84–112 l/ha) of 1,3-D applied broadcast are required in fields heavily infested with the peanut root knot nematode (Dickson and Hewlett, 1988). Also, in such fields, a combination treatment of 1,3-D and a non-fumigant applied post-plant at peg initiation, e.g. aldicarb, surpassed the performance of either applied alone (Rodríguez-Kábana *et al.*, 1985). Metam sodium is used primarily to control CBR; however, it also reduces populations of root knot nematodes (Phipps and Telenko, 2012; Telenko and Phipps, 2012).

Several non-fumigant nematicides were introduced in the late 1950s (Dickson and Smart, 1971; Minton and Morgan, 1974; Sasser *et al.*, 1975a). These older carbamate and organophosphate nematicides are also insecticidal and are formulated as liquids or granules, either of which may be applied in the planting furrow or directly on to the soil surface and incorporated by tillage equipment. The active ingredient depends entirely on water for redistribution; thus, excessive rainfall or irrigation may cause a premature loss of the active ingredient from a root zone. Of the carbamate nematicides currently used in peanut (oxamyl and aldicarb),

aldicarb is among the most effective. Research on aldicarb and oxamyl has been conducted in the USA (Minton and Morgan, 1974; Rodríguez-Kábana *et al.*, 1981, 1982a; Rodríguez-Kábana and King, 1985), India (Singh and Sakhuja, 1984), Australia (Colbran, 1968; Broadley, 1981) and China (Zhang, 1985). In 2015, the fungicide fluopyram was registered for use in the USA for the control of nematodes in peanut. It is currently formulated with the insecticide imidacloprid under the label Velum Total® (Bayer). Fluopyram is a contact nematicide, which, like aldicarb, paralyzes (nematostatic) but does not kill nematodes (Faske and Hurd, 2015). Although published field studies with peanut are limited, fluopyram has shown promise for suppressing root galling by *M. arenaria* and improving peanut yields (Kemerait *et al.*, 2014; Wade *et al.*, 2015).

BIOLOGICAL MANAGEMENT: progress has been made over the past 20 years in the identification of possible biological control organisms that offer exciting possibilities for the future management of plant nematode diseases of peanut. One of these organisms, *Pasteuria penetrans*, is an obligate bacterial parasite of root knot nematodes, reported as a suppressive agent for *M. arenaria* in peanut fields in Florida and Georgia, USA (Minton and Sayre, 1989; Dickson *et al.*, 1991, 1994; Timper *et al.*, 2001). Suppressive soils are defined as those in which disease development is suppressed even though the pathogen is introduced in the presence of a susceptible host (Huber and Schneider, 1982). There are only a few documented reports of plant nematode-suppressive soils, most related to fungal antagonists, while some have reported on suppressive soils infested with *P. penetrans* and root knot nematodes (see Stirling, 2014).

Endospores of *P. penetrans* attach to the cuticle of migrating juveniles (Fig. 11.4) and typically infect after the nematode establishes a feeding site in the root (Stirling, 2014). When endospores of *P. penetrans* were introduced into a soil containing high densities of *M. arenaria*, the bacterium amplified to suppressive levels within 3 years (Oostendorp *et al.*, 1990, 1991; Kariuki and Dickson, 2007) or sooner if high densities of endospores were added (Chen *et al.*, 1996; Mokbel, 2014). Peanut may be an ideal crop for amplifying *P. penetrans* to suppressive densities



Fig. 11.4. Endospores of *Pasteuria penetrans* attached to the cuticle of *Meloidogyne arenaria* (Photograph courtesy of P. Timper.).

because it is grown in a hot climate and is a long-season crop. Both conditions favour the development of *P. penetrans* (Hatz and Dickson, 1992; Serracin *et al.*, 1997). Also, methods for harvesting the peanut crop that include uprooting plants, drying on the soil surface and then collecting pods with a combine harvester leave behind root residues, which will aid in the spread of endospores.

Over a period of 3–5 years, peanut pods and pegs may be totally free of visible galling where *P. penetrans* occurs. Once juvenile densities are reduced, *P. penetrans* density may also diminish, due to a decrease in an available nematode host. Because *P. penetrans* is dependent on its nematode host for reproduction, crop rotations with non-hosts of the nematode lead to reductions in the abundance of the bacterium compared to continuous cropping of peanut (Timper *et al.*, 2001). However, continuous cropping of peanut does not always create highly suppressive conditions. Planting peanut over 4 continuous years in a field site infested with *P. penetrans* increased the percentage of juveniles with endospores and infected females over the 4-year period, but the

level of soil suppressiveness previously reported was not obtained (Cetintas and Dickson, 2005), probably due to *M. javanica* infecting peanut in this field. *M. javanica* was a non-host of the *P. penetrans* isolate in this field. Populations of *P. penetrans* can show a high degree of host specificity even within a species of root knot nematode (e.g. populations of *P. penetrans* may show good attachment to some but not all populations of *M. arenaria*). Even when a *P. penetrans* population exhibits good attachment to a nematode population, not all nematodes within that population are susceptible to attachment (Timper, 2009). Evidence indicates that *P. penetrans* populations contain pathotypes specific to certain genotypes within root knot nematode populations. At a local level, as resistant genotypes of the nematode increase within the population, pathotypes of *P. penetrans* capable of attaching to these resistant genotypes begin to increase, resulting in an ‘arms race’ between the nematode and its parasite (Liu and Timper, 2016).

Methods of diagnosis

SAMPLING: diagnosing root knot nematode damage on peanut can best be undertaken through periodic field observations and examinations of roots, pegs and pods, in conjunction with plant nematode extraction or soil bioassays. A soil bioassay entails growing a root knot nematode-susceptible crop, e.g. peanut, in soil collected from a field site suspected of having harmful nematodes. Characteristic foliage symptoms and galling of underground plant parts are clues that plants are infected by root knot nematodes. To estimate nematode densities in the soil, cores of soil must be taken (Barker *et al.*, 1986). Soil samples should be collected at or near harvest to determine the maximum population density. Soil bioassays are useful for establishing the level of infestations during winter or the early spring months, when population densities are low (Ingram and Rodríguez-Kábana, 1980).

EXTRACTION: *Meloidogyne* juveniles and eggs may be extracted from soil and roots using standard nematological laboratory procedures (see Chapter 4, this volume). Females may be excised from root or pod tissues for identification based on morphology, isozyme phenotypes and

molecular methods (Esbenshade and Triantaphyllou, 1985, 1990; Blok and Powers, 2009).

Determining the relationship of nematode populations to crop loss

A measure of nematode involvement in potential peanut yield losses may be determined by correlating *Meloidogyne* juvenile density in soil or root gall indices with yields in nematicide-treated and -untreated soil. Negative relationships were found between yields and the initial soil population density of *M. hapla* (Rickard *et al.*, 1977) and *M. arenaria* (Dhurj and Vaishnav, 1981; Wheeler and Starr, 1987; Koenning and Barker, 1992), as well as the final soil density of *M. arenaria* (Rodríguez-Kábana *et al.*, 1982b). Root knot damage indices at harvest were correlated with yield for *M. arenaria* and *M. hapla* (Minton and Morgan, 1974).

Based on studies in Texas, more than 10% of the peanut fields from five major producing counties have *M. arenaria* population densities that exceed the level that causes 10% yield suppression (Wheeler and Starr, 1987).

Pratylenchus brachyurus

The lesion nematode *P. brachyurus* is a major nematode parasite of peanut, with a distribution mainly in the warmer peanut production regions of the world (Loof, 1964). The species was first reported on peanut in Alabama, USA, in 1942 (Steiner, 1949), and now affects peanut in most peanut-producing states in the USA and several other countries, including Egypt (Oteifa, 1962), Australia (Colbran, 1968), Zimbabwe (Anon., 1973), South Africa (Venter *et al.*, 1992), Turkey (Kepenekci and Öztürk, 2002), Nicaragua (Augusto *et al.*, 2010) and Ghana (Amponsah and Nutsugah, 2010). Other lesion nematode species, *Pratylenchus coffeae*, *Pratylenchus neglectus* and *Pratylenchus zaeae*, were reported parasitizing peanut in India (Chabra and Mahajan, 1976), Turkey (Kepenekci and Öztürk, 2002) and India (Senthamizh and Sivakumar, 2004), respectively. Taxonomic separation of species of *Pratylenchus* is difficult, because they exhibit little morphological diversity.

Symptoms of damage

Distinct field symptoms of *P. brachyurus* damage on peanut are difficult to discern. Severely infected peanut plants may be stunted and chlorotic, but this is rare. Heavy infection is reported to cause extensive discoloration of below-ground plant parts and reduced root systems and pod weights. Above-ground symptoms may include slight stunting with unthrifty, yellow-green foliage (Good *et al.*, 1958; Miller and Duke, 1961). The most obvious symptom of lesion nematode damage on peanut is small, purplish-brown to black lesions that form on the peanut shell (Fig. 11.5) (Good *et al.*, 1958). The nematode-induced lesions are described as giving pods a speckled appearance, and are conspicuous to the trained eye (Miller and Duke, 1961). These lesions have distinct boundaries and first appear as small brown tunnels in the shell, which can later coalesce to form larger lesions. When coalesced, they are difficult to separate from those induced by other soil microbes. Secondary soil-borne pathogens may enter these lesions, causing them to increase in size, or the infected pegs and pods may rot. Infection of pegs by *P. brachyurus* has been correlated with a peg rot condition resembling the peg rot disease caused by *S. rolfsii* (Good *et al.*, 1958). Combinations of fungal- and nematode-induced lesions may occur, but this has received little study



Fig. 11.5. Lesions on groundnut pods caused by *Pratylenchus brachyurus* infection. (Photograph courtesy of N. Minton.)

(Good *et al.*, 1958). *P. brachyurus* can be found in roots and pegs, as well as shells of mature pods, but the nematode is more numerous in shell tissue.

Symptoms of pod lesions may vary depending on the type of peanut or cultivar; for example, they may be less conspicuous on Virginia-type peanut than on Spanish and runner types (Good *et al.*, 1958; Minton *et al.*, 1970). *P. brachyurus* feeding within the pegs weakens them, resulting in pod loss at harvest (Good *et al.*, 1958; Jackson and Sturgeon, 1973). Also, microorganisms that colonize damaged pods may penetrate the shell and damage the seed; thus, the yield, as well as the quality and value of the crop, may be reduced (Good *et al.*, 1958).

Biology and life history

P. brachyurus is a migratory endoparasite that infects peanut roots, pegs and pods, and feeds within the parenchymatous tissues. All life stages of the nematode, except the egg, are infective. All life stages are found within parasitized plant tissue, and the second-, third- and fourth-stage juveniles, and adults are fusiform shaped. They remain mobile and are capable of migrating within plant tissue. The nematode may penetrate anywhere along roots, pegs and pods and move from old infection sites to induce new infection sites. The nematode reproduces rapidly, which results in roots, pegs and pods containing thousands of nematodes; however, few lesion nematodes will be detected in the soil surrounding roots. Females lay eggs singly inside lesions or outside the plant tissue in soil. Apparently, the pod shell tissue is more favourable for reproduction, with 6–8 times greater numbers occurring in it as compared with equal portions of root tissue; in mature shells, the high densities of *P. brachyurus* form dark-coloured necrotic lesions (Good *et al.*, 1958). The nematodes remain viable in these infected shells and serve as a source of inoculum even after the shells have been cured and stored over winter. Irrigation events markedly increase lesion nematodes in peanut pegs (Good and Stansell, 1965).

The presence or absence of host races within *P. brachyurus* has not been documented, although their existence has been suggested due to variation in numbers extracted from the roots of citrus seedlings when inoculated with different

nematode isolates (O'Bannon and Tomberlin, 1970). Field observations have also indicated behavioural differences within *P. brachyurus* populations, particularly on peanut (Payan and Dickson, 1988). However, attempts to separate races of *P. brachyurus* failed to discern behavioural differences among four populations tested on seven species of crop plants (Payan and Dickson, 1988). The densities of two nematode populations from peanut on these seven crop plants were not different from two other nematode populations that originated from soybean or maize.

Survival and means of dissemination

P. brachyurus may overwinter in peanut plant debris remaining in the soil (Good *et al.*, 1958; Feldmesser and Rebois, 1965). Since the nematode is polyphagous, it may survive and overwinter in live roots of many winter crops and weeds, as well as in dead tissues. In South Africa, 66% of *P. brachyurus* from potato and maize were found in the soil organic matter at the end of winter, although the organic matter constituted only 0.3% of the soil (Koen, 1967). *P. brachyurus* was recovered from peanut shells that were stored at 24°C for 3, 6 and 28 months (Boswell, 1968).

P. brachyurus may be disseminated in many of the same ways as *Meloidogyne* spp. Since this is a migratory endoparasite, it can be transported in infected roots and other underground plant parts in the soil. Generally, the major method of spread is by human activity, involving the movement of plant material and soil and tillage equipment. Peanut shells that are used to mulch soil may carry live nematodes (Good *et al.*, 1958). Also, water movement across infested fields due to either rainfall or irrigation may transport the nematode.

Environmental factors affecting parasitism

The distribution of and parasitism by *P. brachyurus* are temperature related; consequently, the nematode is restricted to warmer regions of the world (Loof, 1964). Reproduction in root and shell tissue of peanut was greatest at 26°C (Boswell, 1968). Soil types and moisture may also affect the parasitism of peanut by *P. brachyurus* (Endo, 1959; Good and Stansell, 1965).

Disease complexes

Disease complexes involving *P. brachyurus* and other soil microorganisms that would produce a peg rot have been suggested (Good *et al.*, 1958). *P. brachyurus* and *S. rolfii* were frequently found occurring together as pathogens. Lesions of peanut pods were found to contain both *P. brachyurus* and mycelium of fungi, most notably *Rhizoctonia solani*, *Fusarium* spp. and *Penicillium* spp. (Boswell, 1968). It is reported that lesions on roots, pods and pegs allow fungi and bacteria to enter damaged cells (Jackson and Sturgeon, 1973). However, the suppression of *P. brachyurus* in peanut by aldicarb treatment had no effect on the level of pod rot, indicating independence between nematodes and pod rot pathogens (Augusto *et al.*, 2010). There is some indication that the presence of *P. brachyurus* is related to an increase of *A. flavus* in peanut shells but not in seeds (Jackson and Minton, 1968).

Economic importance and population damage threshold levels

P. brachyurus is only occasionally associated with severe peanut yield loss; thus, damage by this nematode is often overlooked. Consequently, damage estimates for this nematode may be low, since it has been reported in a large percentage of the peanut production areas in the USA and in other countries. Reports of the percentages of fields sampled that were infested by *P. brachyurus* in the USA include: Alabama 84% (Ingram and Rodríguez-Kábana, 1980); Georgia 17% (Motsinger *et al.*, 1976); Texas 16% (Wheeler and Starr, 1987); and South Carolina 14% (Alexander, 1963). Damage has also been reported in Florida (Dickson and Smart, 1971) and Arkansas (Jackson and Sturgeon, 1973). In Egypt, it was found that *P. brachyurus* infested 81% of peanut fields (Oteifa, 1962), but in a later survey only 10% of samples were found to contain the nematode (Ibrahim and El-Saedy, 1976a). *P. brachyurus* occurs in peanut fields in a variety of soils in South Burnett, Australia (Colbran, 1968); it was also widespread throughout Atherton Tablelands in north Queensland, Australia, and was absent only in soils that had recently been brought into cultivation (Broadley, 1981). *Pratylenchus* sp. was found in 5000 soil samples collected from peanut fields in Guyana (Singh, 1972).

Damage thresholds for *P. brachyurus* have not been well defined. Densities of *P. brachyurus* per gram of shell have been correlated with yields (Good *et al.*, 1958; Minton and Morgan, 1974). A significant yield increase was reported in fumigated plots in which there were 242 *P. brachyurus*/g shell, as compared with the untreated plots that had 2771/g shell (Boswell, 1968). In Georgia, a significant yield increase was obtained in fumigated plots in which there were 127 *P. brachyurus*/g shell compared with 2280/g in untreated plots (Minton and Morgan, 1974). Similarly, a significant yield increase was observed in Nicaragua in aldicarb-treated plots, with 124 *P. brachyurus*/g shell compared to plots without nematicide with 458/g shell (Augusto *et al.*, 2010). Damage in the field is probably caused by high initial densities that carried over from a previous crop, e.g. maize. In Texas, USA, the nematode did not increase on cotton or soybean cultivars (Thames, 1982). However, in a greenhouse study, cotton was a better host for *P. brachyurus* than was peanut or maize (Timper and Hanna, 2005).

Management

Potential yield losses on peanut caused by *P. brachyurus* are considered to be relatively small in relation to the amount of peanut acreage infested. Few management tactics specifically targeting this nematode are therefore employed, except in certain areas where severe infestations and crop losses are known to occur. Growers who produce peanut for green boiling or roasting in the shell generally must seek fields known to be free of *P. brachyurus*. For such crop uses, unsightly blemishes on the shells caused by lesion nematode are not acceptable.

CULTURAL PRACTICES: generally, crop rotations for the management of *P. brachyurus* in peanut are not effective, because of its wide host range that includes many agricultural crops and weeds, and because there are few alternative cash crops available for use in rotations with peanut (Endo, 1959; Koen, 1967). Densities of *P. brachyurus* were greater in maize than in peanut in a maize-peanut rotation (Good *et al.*, 1954). *P. brachyurus* was also present in soil following rotations with lupin (*Lupinus hirsutus*), oat (*Avena sativa*) and native grasses. However,

densities were greater in lupin than in oat or native grasses (Good *et al.*, 1954). Fallowing for 6 weeks (May–June) or 9 months (May–March) in the southern USA reduced population densities of *P. brachyurus* in soil to undetectable levels (Brodie and Murphy, 1975).

Timely harvesting increases the yield and value of peanut in fields heavily infested with *P. brachyurus* (Good *et al.*, 1958; Boswell, 1968). However, larger peanut yields were reported from *P. brachyurus*-infested soil that had been fumigated, irrigated and harvested earlier than normal than for non-irrigated peanut (Good and Stansell, 1965). Although irrigated peanut yielded more, *P. brachyurus* was tenfold more numerous in shell tissue in irrigated than in non-irrigated plots.

RESISTANCE: no commercial peanut cultivar possesses useful levels of resistance to *P. brachyurus*. Six peanut cultivars were reported to be equally infected with *P. brachyurus*, but lesion symptoms were not as conspicuous on two of them (Minton *et al.*, 1970). Two peanut plant introductions, PI290606 and PI295233, were reported to be resistant (Smith *et al.*, 1978). This work was confirmed and an additional resistant plant introduction, PI365553, was identified (Starr, 1984).

CHEMICAL: where severe infestations of lesion nematodes occur, nematicide applications may be beneficial. Nematicides that control *Meloidogyne* spp. also control *P. brachyurus* (Good and Stansell, 1965; Jackson and Sturgeon, 1973; Minton and Morgan, 1974).

Methods of diagnosis

SAMPLING: both soil and below-ground plant parts can be assayed to determine population densities of *P. brachyurus*. Soil samples should be collected in such a manner as to obtain roots, pegs and pods. Shells usually yield more *P. brachyurus* per unit weight of tissue than roots. Soil samples should be collected shortly before or after harvest when soil population densities are likely to be at their greatest. The use of bioassays to establish the level of infestation in soils may be helpful if samples are collected during the winter or early spring when population densities are low.

EXTRACTION: *P. brachyurus* adults and juveniles may be extracted from roots by incubating the roots in a mist chamber, and from soil using standard nematological extraction procedures (see Chapter 4, this volume).

Belonolaimus longicaudatus

The sting nematode *B. longicaudatus* is an important ectoparasite that is highly virulent on a wide range of crop plants grown in soils with a high sand content. *B. longicaudatus* was first described from Florida, USA (Rau, 1958), where the species is considered among the state's most important soil-borne plant pathogens. The host range of *B. longicaudatus* is extensive and includes agronomic, horticultural, ornamental and tree crops (Perry and Rhoades, 1982; Smart and Nguyen, 1991; Bekal and Becker, 2000; Han *et al.*, 2006). The nematode may be encountered fairly frequently in south-eastern USA, especially along the Atlantic and Gulf coastal regions (Smart and Nguyen, 1991). There are a few other occurrences found in the midwestern region of the USA, where deep, sandy soils exist. The species was also reported from California, where it was found infecting golf greens on a single golf course (Mundo-Ocampo *et al.*, 1994). The species has been reported outside the USA only in Bermuda, Brazil, Costa Rica, Mexico and Puerto Rico.

Although sting nematodes are prevalent in well-drained sandy soils in most states where peanut is commonly grown, only a small percentage of the peanut acreage is reported to be damaged by this nematode (Holderman, 1955; Rau, 1958; Wheeler and Starr, 1987; Kutsuwa *et al.*, 2015). *B. longicaudatus* caused yield reduction of peanut in Virginia, North Carolina, Oklahoma, and Florida (Sasser *et al.*, 1975b; Anon., 1987; Kutsuwa *et al.*, 2015), but not in Georgia (Motsinger *et al.*, 1976), the USA's largest producer of peanut. There are no reports of *B. longicaudatus* affecting peanut grown outside the USA.

In some instances, the nematode has been observed to cause damage to young peanut seedlings only to see the problem diminish as the season progresses. Why this happens is poorly understood. However, in other instances, the nematode is capable of causing severe damage

to peanut over the entire crop season (Cooper *et al.*, 1959; Sasser *et al.*, 1967; Kutsuwa *et al.*, 2015).

Symptoms of damage

B. longicaudatus is an ectoparasitic nematode that feeds at root tips and along succulent roots, as well as on young pegs and pods. The most obvious field symptom is small to large irregular patches of dwarfed, chlorotic seedlings and a reduced plant stand. Once seeds have germinated, the young seedlings may be killed if infected with large numbers of sting nematodes. Infected plants may exhibit an abnormal root system; sting nematodes cause a cessation of root growth that at times can be extreme. The root damage may be visible on roots along the stem at ground level, along only a portion of the main roots or, in the most severe cases, along the entire root system. In addition, if pegs and pods have formed, they appear with numerous small, round punctate-like lesions (Fig. 11.6). The yield and quality of peanut may be severely reduced.

Host races

The existence of physiological races of *B. longicaudatus* with different host ranges has been suggested (Abu-Gharbieh and Perry, 1970; Robbins and Barker, 1973). Additionally, populations of *B. longicaudatus* from North Carolina and Georgia differed morphologically from each other, and from the original description of *B. longicaudatus* (Robbins and Hirschmann, 1974). Matings

between these two populations resulted in a few non-fertile offspring, indicating that these populations might be different species. The nematode's taxonomic status has been additionally questioned because of reported variations in morphology and host specificity among different isolates (Perry and Norden, 1963; Good, 1968; Abu-Gharbieh and Perry, 1970; Rau and Fassu-liotis, 1970; Robbins and Barker, 1973; Gozel *et al.*, 2006). For example, a population of *B. longicaudatus* collected from a peanut-growing region in north-central Florida did not cause damage or reproduce well on peanut, whereas a population from a non-peanut-growing region in central Florida did (Perry and Norden, 1963). Populations of this nematode are definitely pathogenic on peanut in Florida (Kutsuwa *et al.*, 2015), North Carolina and Virginia (Cooper *et al.*, 1959).

Survival and environmental factors affecting parasitism

The rather specific and limited distribution of *B. longicaudatus* indicates that soil type and perhaps other biological and environmental factors constrain its distribution. Soil texture, soil temperature and moisture are critical for the nematode's development (Perry, 1965; Robbins and Barker, 1974). Fine-textured soils are believed to inhibit its movement and reproduction. There is minimal reproduction in soils with <80% sand content or >10% clay content (Robbins and Barker, 1974). In Virginia, *B. longicaudatus* is found only in the A-horizon of soils with a sand content of 84–94% (Miller, 1972). Greater densities of this nematode were also reported in the upper 20 cm of soil compared with a 20–40 cm depth, even though both layers contained 94–95% sand (Perez *et al.*, 2000).

Soil temperature and moisture have a large influence on the life cycle and reproductive rate in Florida, Georgia and North Carolina populations (Boyd and Perry, 1971; Robbins and Barker, 1974; Smart and Nguyen, 1991). In Florida, *B. longicaudatus* reproduced better at 29°C than at 27°C, but was greatly reduced at 35°C (Boyd and Perry, 1971). The reproduction of the Georgia population was greatest at 30°C, whereas reproduction of the North Carolina population was reduced at 30°C (Robbins and Barker, 1974). In Florida, populations either die or migrate downwards



Fig. 11.6. Peanut pods with punctate lesions caused by *Belonolaimus longicaudatus*. (Photograph courtesy of D. Dickson.)

when soil temperatures at 2.5 cm below the bare soil surface reach 39.5°C or higher (Boyd and Perry, 1971). The optimum soil moisture for reproduction was reported to be 7% (Robbins and Barker, 1974). In a greenhouse study, the life cycle of *B. longicaudatus* was completed in about 28 days (Smart and Nguyen, 1991), but in excised maize root cultures held at 28°C, the timespan from egg to adult varied depending on the site of origin for five populations (Han *et al.*, 2006). A population from Georgia completed its life cycle in the shortest time at 18 days, and one from North Carolina required 25 days, while three Florida populations were intermediate. A California population of *B. longicaudatus* completed its life cycle in 1 month at 26–27°C and 24 days at 28°C (Huang and Becker, 1997). All juvenile stages, as well as the adults, fed on root meristems, with mating occurring after males and females completed their last moult (Han *et al.*, 2006).

Economic importance and population damage threshold levels

Economic losses for peanut in the USA due to *B. longicaudatus* appear small, despite the extreme damage this nematode is capable of inflicting. Statewide, yield losses caused by sting nematodes in peanut are reported only for North Carolina (0.38%), Oklahoma (0.25%) and Virginia (0.50%) (Anon., 1987). Increases in yields by as much as 109–400% compared with untreated controls were obtained in North Carolina nematicide trials in which the average population density of *B. longicaudatus* ranged from 10 to 43 nematodes/100 cm³ soil (Cooper *et al.*, 1959; Sasser *et al.*, 1960). The economic threshold level varied from 2 to 5 *B. longicaudatus*/130 cm³ soil, depending on the nematicide used. In Florida, the average dry weight of peanut kernels harvested from field sites without the sting nematode was 2.74-fold higher than those harvested from a site infested with the nematode (Kutsuwa *et al.*, 2015). Yield was depressed by an estimated 64% where sting damage was observed.

Management

No commercial peanut cultivar is resistant to *B. longicaudatus*. Due to its wide host range, it is difficult to devise a satisfactory crop rotation plan for the sting nematode. Only a few crop

plants, such as tobacco (*Nicotiana tabacum*) and watermelon (*Citrullus lanatus*), have been shown to reduce densities when grown in rotation with peanut (Holderman and Graham, 1953; Bailey, 1988). The application of nematicides is an option where sting nematode densities are above the economic threshold. Although both fumigant and non-fumigant nematicides have provided sting nematode control and resulted in increased peanut yields compared with non-treated peanut (Cooper *et al.*, 1959; Sasser *et al.*, 1960, 1975b), the chemistries used in those field trials are no longer marketed. The nematicide chemistries that are currently labelled for use on peanut, although not specifically tested on sting nematode, would most likely provide control.

Methods of diagnosis

SAMPLING AND EXTRACTION: early season seedling damage by *B. longicaudatus* is apparent, especially if densities are high. Above-ground symptoms will include severely stunted plants that appear in scattered portions of the field, and the examination of roots of seedlings for damage, as well as assessments of densities in the soil, are suggested. Soil samples should be collected using the procedures recommended for recovery of ectoparasitic plant nematodes (see Chapter 4, this volume). The extraction of *B. longicaudatus* from soil may be undertaken using any one of a number of standard extraction procedures.

Determining the relationship of populations to crop loss

The effects of *B. longicaudatus* on peanut are reflected in plant growth, yield and quality (Cooper *et al.*, 1959; Sasser *et al.*, 1975b; Kutsuwa *et al.*, 2015). Significant negative correlations of soil nematode density with yield and growth may be obtained during most of the growing season.

Aphelenchoides arachidis

The testa nematode *A. arachidis* was described from northern Nigeria from peanut (Bos, 1977a,b). This nematode was also reported on peanut pods of three cultivars in Egypt (Montasser *et al.*, 2008), in

soil samples from Uganda and in both pods and testae of peanut from the Vaalharts Irrigation Scheme, Northern Cape Province in South Africa (Lesufi *et al.*, 2015).

Symptoms

A. arachidis is a parasite of pods, testae, roots and hypocotyls, but not the cotyledons, embryos or other parts of the plant (Bos, 1977a; Bridge *et al.*, 1977). Seed coats were discoloured when more than 2000 *A. arachidis*/testa were present (Bridge *et al.*, 1977). Heavily infected seeds, examined immediately after removal from fresh, mature pods, are a light brown, have translucent testae and dark vascular strands within the testae. After infected seeds are dried, testae are often wrinkled, and are darker brown than in non-infected seeds. Nematodes are found mainly in the subepidermal parenchymatous layer, where walls are broken and cells enlarge, and around the tracheids of the testa. Testae infected with *A. arachidis* are thicker and more uneven than normal testae. The epidermal layer of the seed coat is reduced in infected testae, and the basal tissue, including the aleurone layer, is disorganized. Infected seeds of cv. Spanish 205 weighed less than healthy seeds, but nematode damage has little effect on seed germination.

Biology and life cycle

The nematode is a facultative endoparasite of peanut (Bridge *et al.*, 1977). It also feeds ectoparasitically on peanut roots and on two fungi, *Macrophomina phaseolina* and *Botrytis cinerea*, which have been associated with seeds on agar plates. *A. arachidis* were found in the parenchymatous tissues of the testa, root cortex and hypocotyl, but not in the central stele or vascular bundles (Bridge *et al.*, 1977). Pods are invaded 10 days after the fruiting pegs penetrate into the soil, but the number of nematodes in pods does not increase rapidly until after 30 days, with the largest densities present at about day 60. All stages of the nematode, including eggs, were found throughout the testae, but at the end of the growing season, heavily infected testae of mature seeds contained mainly juvenile stages, with few adults. Testae showing no external symptoms contained mostly adults and eggs, often arranged along the vascular elements of the seed coats.

Biotypes

It is suggested that there are two biotypes of *A. arachidis*, one occurring on cereals and one on both cereal and peanut (Bos, 1977b).

Survival and means of dissemination

A. arachidis can survive desiccation in stored peanut pods for 12 months (Bridge *et al.*, 1977). The second-, third- and fourth-stage juveniles were extracted from dried testae and shells, with no particular stage predominating, but adults were found alive only occasionally in either the testae or shells of stored pods. No active nematodes were extracted from infected pods sundried in the field before storage. Volunteer peanut plants in an infested field contained many adult nematodes, which indicated that they continued to develop to maturity under natural conditions in pods left in the ground during the dry season in Nigeria. Unless appropriate precautions are taken, *A. arachidis* has the potential to become a serious pest worldwide, because it can be disseminated readily in infected seeds (Bridge *et al.*, 1977).

Disease complexes

The infection of peanut by *A. arachidis* in field experiments predisposed seeds to invasion by fungi (McDonald *et al.*, 1979). Nematode-infected seeds had higher levels of fungal infection (*R. solani*, *S. rolfsii*, *M. phaseolina* and *Fusarium* spp.) than those that appeared to be nematode free. Fungi isolated from the South African *A. arachidis*-infected peanut seeds were identified as *Thielaviopsis basicola* and *Necocosmospora vasinfecta* (Lesufi *et al.*, 2015). Both seedling emergence and total emergence rates were lower for nematode-infected seeds than for healthy seeds.

Economic importance and population damage threshold levels

Peanut yields are not affected by *A. arachidis*; however, peanut is devalued by the nematode because the nematode causes shrivelled and discoloured seeds (Bridge *et al.*, 1977). Severe infection of peanut with *A. arachidis* not only has an adverse effect on the appearance and size of seed but also it may predispose seeds to an invasion

by fungi, which may lead to reduced seed emergence (McDonald *et al.*, 1979). With at least four reports of *A. arachidis* occurring outside Nigeria, it appears that this nematode is either spreading or is more widespread than previously determined (Lesufi *et al.*, 2015), and could become a major economic pest.

Management

Only limited information is available on the management of *A. arachidis* on peanut. No field-applied treatments have been reported, but a number of preventive measures are effective against further spread of the nematode. Immersing infected seed in four times their volume of water heated to 60°C and allowing to cool for 5 min provides complete control of the nematode without affecting germination (Bridge, 1975; McDonald and Misari, 1976; Bridge *et al.*, 1977). In northern Nigeria, very dry conditions make it possible to sun-dry pods after harvest in order to reduce nematodes in pods (Bridge *et al.*, 1977). In more humid areas, the sun-drying of pods may not be effective. Shelling peanut before planting will eliminate the tissues in which most nematodes occur and survive best (Bridge *et al.*, 1977).

Tylenchorhynchus brevilineatus

T. (syn. *Bitylenchus*) *brevilineatus* was first observed causing damage to peanut in 1976 in the Kalahasti area of Andhra Pradesh State, India (Reddy *et al.*, 1984). The disease caused by this nematode is known as 'Kalahasti malady'. Since 1976, the disease has been widespread in the Kalahasti area, but has also been observed in Nellore District in Andhra Pradesh (Reddy *et al.*, 1984). This nematode has not been reported as a pathogen of peanut elsewhere.

Symptoms of damage

Disease symptoms are characterized by small pods and a brownish-black discoloration of pod surfaces (Reddy *et al.*, 1984). Small, brownish-yellow lesions appear on the pegs and pod stalks and on young, developing pods. Lesion margins are slightly elevated because of the host cell proliferation around them. The length of pod stalks is greatly reduced, and in advanced

stages the pod surface becomes completely discoloured, but seeds from diseased pods are unaffected. Discoloration is also observed on roots, but is less severe than on pods.

Pathogenicity tests in the greenhouse corroborated field observations (Reddy *et al.*, 1984). Peanut plants inoculated with 500 *T. brevilineatus*/12 cm diameter pot were severely stunted and had reduced root systems. Lesions were present on the roots but were not extensive. Pods were severely discoloured and small, but seeds from the discoloured pods were undamaged. Brownish-yellow lesions were observed on individually inoculated pods after 15 days. The number of lesions increased and extensive discoloration was observed 30 days after inoculation. Yield suppression of 60–70% has been reported (Rao *et al.*, 1986).

Management

The non-fumigant nematicides aldicarb and carbofuran provided control of *T. brevilineatus* when applied to peanut 20 days post-plant. These nematicides reduced soil population densities of *T. brevilineatus* and the percentage of diseased pods, and increased plant height, pod yield and pod and kernel weights (Reddy *et al.*, 1984). However, nematicides may not be cost-effective for many peanut farmers (Rao *et al.*, 1986).

Crop rotation or intercropping with poor or non-hosts of *T. brevilineatus* is effective in reducing the severity of Kalahasti malady. When grown before peanut, rice and sunn hemp reduced densities of the nematode and improved pod yields compared to continuous peanut (Naidu *et al.*, 2000a). Similarly, when intercropped with peanut, marigold and mustard reduced numbers of *T. brevilineatus* and increased pod yield over peanut alone (Naidu *et al.*, 2000b). Crops grown in rotation with peanut, however, must be selected with care because many, such as sugarcane (*Saccharum officinarum*), castor (*R. communis*), wheat (*Triticum aestivum*), mung bean (*Vigna radiata*) and maize (*Z. mays*), can support population increases of *T. brevilineatus* (Gupta, 1986; Thakar *et al.*, 1986; Naidu *et al.*, 2000a).

In 1990, the first peanut cultivar with a high level of resistance to *T. brevilineatus*, 'Tirupati 3', was released in India (Mehan *et al.*, 1993; Vasanthi *et al.*, 2011). Tirupati 3, a Virginia bunch variety, was not widely planted because

farmers preferred a faster-maturing peanut. This cultivar was subsequently crossed with a shorter-season Spanish bunch cultivar, to obtain 'Kalahasti'. Kalahasti is a short-season, high-yielding peanut cultivar with resistance to *T. brevilineatus* (Vasanthi *et al.*, 2003). Two SSR primers were associated with resistance to *T. brevilineatus* in peanut, which should greatly improve screening germplasm for resistance to this nematode (Vasanthi *et al.*, 2011).

Ditylenchus africanus

The peanut pod nematode *D. africanus* was originally described as *Ditylenchus destructor*, the potato rot nematode. It was first reported damaging peanut in the Transvaal Province of South Africa in 1987 (Jones and De Waele, 1988). A subsequent survey revealed the presence of this nematode in seven major peanut-producing regions (De Waele *et al.*, 1989), where 75% of 877 seed samples that graded 'damaged' were infected. An average of 160 nematodes/seed was recovered. This nematode has not been reported on peanut in other parts of the world.

Symptoms of damage

D. africanus can survive in low densities on weeds (De Waele *et al.*, 1990) and crops other than peanut (Basson *et al.*, 1990), but causes damage only to peanut (De Waele *et al.*, 1989). It occurs on roots, pegs, shells and peanut seeds (De Waele *et al.*, 1989), and while visible symptoms are not apparent on roots, seeds show blemishes and germinate prematurely before harvest (Fig. 11.7). Infected pods of cv. Sellie are

black, resembling black pod rot or black hull caused by the fungus *Chalara elegans*. Approximately 40–60% of the pods and seeds are destroyed in heavily infested fields (Jones and De Waele, 1988). *D. africanus* is present in both hulls and seeds, which results in a lower-quality grade (McDonald *et al.*, 2005) and reduced peanut yield (Jones and De Waele, 1990; Venter *et al.*, 1991).

In greenhouse pathogenicity tests (De Waele *et al.*, 1989), nematodes were present in the peg, exocarp and endocarp, testa and embryo, and on the cotyledons. The first symptom to develop was brown necrotic tissue at the pod base at the juncture with the peg. The surface of infected tissue is dark brown, with a corky appearance. The most distinct symptom of advanced disease is dark brown to black discoloration of veins that extend longitudinally in the exocarp just beneath the pod surface. Infected pods lack the lustre of healthy pods and appear dead. Infected seeds are usually shrunken, and the micropyles are dark brown to black. The testae are flaccid, have dark vascular strands and are removed easily. The inner layer of the testa has a distinct yellow discoloration. Infected embryos are usually olive green to brown, instead of having the normal colourless to yellow appearance.

Biology and life history

D. africanus develops from egg to adult in 8 days at 25°C. At 28–30°C, egg hatching starts at around 3 days (De Waele and Wilken, 1990). By the 6th day, 90% of eggs have hatched. The nematode is able to enter a state of anhydrobiosis, with about one-third of the anhydrobiotic

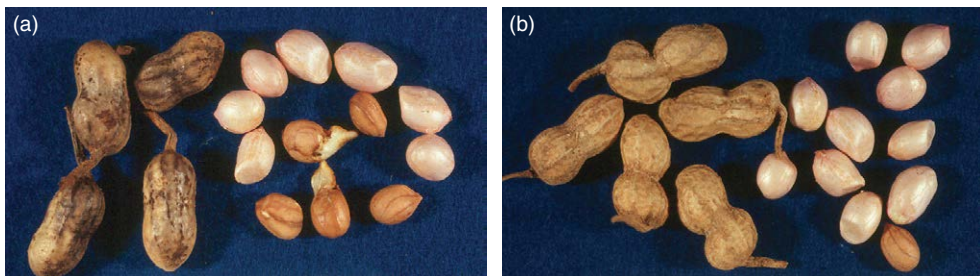


Fig. 11.7. Peanut pods infected with *Ditylenchus africanus* (a) showing lesions and premature germination of seed, and (b) healthy, uninfected shells and seed. (Photograph courtesy of S. Steenkamp.)

nematodes becoming active after rehydration to invade the hulls and seeds of a newly planted crop (Basson *et al.*, 1993). Although *D. africanus* is present in the roots of peanut and in soil, 90% of the total population at harvest is found in the pods (Basson *et al.*, 1991). Infections up to 97,000 nematodes/pod are not uncommon.

D. africanus enters the immature pegs and pods of peanut at the peg connection; however, the infective stage is unknown (Jones and De Waele, 1990). It subsequently invades the parenchymatous region of the hull exocarp and the endocarp, and eventually the seed testa. The nematode causes malformation of the cells of infected tissues, cell wall breakage and cell collapse (Venter *et al.*, 1995). Damage appears to be caused by enzymatic activity. The entire parenchyma region in some testae is destroyed. In immature pods, *D. africanus* may move across the fibrous region of the mesocarp into the hull of the endocarp. In mature pods, the lignification of the fibrous mesocarp at around 105 days is a barrier to penetration of the inner pod tissues (Venter *et al.*, 1995). Nematodes artificially inoculated after 105 days are no longer able to cause damage to the seed. Increased numbers of eggs and anhydrobiotic forms of the nematode are found in the hull tissues, and eggs are found in the seed testae of late harvested pods (~189 days after planting). Eggs, some containing first-stage juveniles, have been observed in the parenchymatous mesocarp of the hull. Also, egg densities increase in the seed testa of late harvested pods. Both occurrences may indicate the onset of survival mechanisms of the nematode. Apparently, all life stages can be found in the hull. It appears that eggs and anhydrobiotic forms are involved in winter survival in decaying hulls and stubble, whereas eggs are the important survival stage in stored seed (Basson *et al.*, 1993).

Economic importance

About 200,000 ha of peanut are grown annually in South Africa, with *D. africanus* present in all major peanut production areas of the country. Greenhouse damage studies demonstrate that at 250 nematodes/3-l pot, 10–25% of seeds germinated prematurely before harvest, and the fresh weight of harvested seed was reduced 20–50%. The effect of *D. africanus* on peanut is, however, primarily qualitative, resulting

in the downgrading of peanut consignments and a lower income for the producer (McDonald *et al.*, 2005).

Management

D. africanus is difficult to control. Nematicides registered in South Africa for the control of nematodes on peanut were often ineffective in sufficiently reducing the *D. africanus* population because of its short life cycle and high viability of the eggs (De Waele and Wilken, 1990; Basson *et al.*, 1992). The cultivation of certified, nematode-free seed has been hampered by the omnipresence of this nematode over the whole peanut production area (De Waele *et al.*, 1989), the inefficiency of nematicides (Basson *et al.*, 1992) and the unavailability of *D. africanus*-resistant peanut cultivars on the market (Basson *et al.*, 1991). Sources of resistance have since been identified, but need to be incorporated into commercial cultivars (Steenkamp *et al.*, 2010, 2016). The use of crop rotation is also not effective, because *D. africanus* can survive in low density on crops used in rotation with peanut (Basson *et al.*, 1990) and reinfect and damage a subsequent peanut crop, even from low initial densities (Basson *et al.*, 1992). A promising alternative for the management of *D. africanus* is the use of *D. africanus*-resistant peanut cultivars.

Other nematodes

Aphasmatylenchus straturatus was described in 1970 from around the roots of peanut in southwest Burkina Faso, West Africa, near Niangoloko village (Germani, 1970). It has not been reported to occur outside of Burkina Faso. *Scutellonema cavenessi* was described from northern Nigeria (Sher, 1964), but has since been found associated with most cultivated plants in Senegal and Mali. In Senegal, *S. cavenessi* was associated with poor growth of peanut (Germani, 1979, 1981). The ring nematode *Mesocriconema ornatum* occurs in a large percentage of the peanut production regions of the USA, and has been reported in a few countries in Africa. Even though *M. ornatum* is a weakly pathogenic nematode, large densities of this nematode may damage peanut. Additional information on *A. straturatus*, *S. cavenessi* and *M. ornatum* is available in Dickson and

De Waele (2005). A worldwide list of nematode pathogens associated with peanut has been compiled (Sharma, 1985). The list is extensive and includes many genera and species that have not been proven to cause economic damage to peanut. Additional research may demonstrate that some of these species are, in fact, pathogenic and pose a serious threat to peanut production, while others may feed on peanut but cause little or no economic damage.

The possibility that two nematodes that are not considered serious pathogens of peanut interact with a virus to cause disease has been suggested. The clump disease of peanut, caused by a virus, was eliminated in Senegal by treating the soil with a nematicidal fumigant (Merny and Mauboussin, 1973). It was suggested that one or more nematodes were acting as a vector and indicated that *Longidorus pisi* was present in soil samples. In India, the disease was reduced in field experiments by 97 and 84% with 1,2-dibromo-3-chloropropane (DBCP) and aldicarb, respectively (Singh and Sakhuja, 1984). Soil samples collected from the rhizosphere of diseased plants always contained *Paralongidorus citri*. Both nematodes are capable of transmitting plant viruses.

Nematodes of Bambara Groundnut

Bambara groundnut is primarily parasitized by the root knot nematodes *M. javanica* and *M. incognita* (Ogbuji, 1979). Crop damage by *Meloidogyne* spp. has been reported in Malawi, Zimbabwe, South Africa, Botswana, Nigeria and Ghana (Martin, 1959; McDonald and De Waele, 1989; Kwerepe and Labuschagne, 2004; Jada *et al.*, 2011; Kankam and Adomako, 2014). Symptoms of infection include severe galling of the roots, stunting and yellowing of the plants, and reduced root systems, plant density and pod yield (McDonald and De Waele, 1989).

Several approaches have been used to manage root knot nematodes in Bambara groundnut. Most cultivars and genotypes of Bambara groundnut are susceptible to these nematodes; however, a few genotypes in South Africa were more tolerant than other genotypes to the root knot nematodes (Ogbuji, 1979; McDonald and De Waele 1989). Of 50 landraces from Botswana and South Africa, Kwerepe and Labuschagne

(2004) identified one with resistance and three with tolerance to *M. incognita* race 2. In a field heavily infested with *Meloidogyne* spp., 13 of 250 landraces had a low incidence of plants with root galls (<15%) compared with 128 of the landraces having >60% of plants with galls (Asiwe, 2009). These resistant landraces provide a good resource for developing nematode-resistant cultivars. Other management options may be effective in reducing nematode densities, but may not be economical for small producers. Carbofuran, applied at planting, reduced galling and improved yields of Bambara groundnut by three- to fourfold (Jada *et al.*, 2011). Neem seed powder, applied twice early in the season, reduced root galling and improved yields by 20% (Fankam and Adomako, 2014). Biofumigation with *Brassica* spp. was also effective in reducing root galling in Bambara groundnut under glass-house conditions; however, higher application rates were phytotoxic (Kwerepe and Labuschagne, 2003). The most effective treatment was a combination of biofumigation followed by solarization.

Conclusions and Future Prospects

Potential peanut yield losses due to plant nematodes occur in every major peanut production region of the world. With losses estimated at 12% (Sasser and Freckman, 1987), it is clear that improved strategies are badly needed to reduce these losses.

Meloidogyne spp. are the most important plant nematodes damaging peanut in most regions of the world, but in some regions, such as in West Africa, other species may be more serious. A number of nematodes, such as *A. arachidis*, *A. straturatus*, *S. cavenessi*, *T. brevilineatus* and *D. africanus*, cause serious damage to peanut in isolated regions of Africa and Asia, but not in other regions of the world. *B. longicaudatus* is a pathogen of peanut in only certain regions of the USA. Questions have been raised as to why these nematodes have been reported damaging peanuts specifically in these areas alone, and what the probability is of their becoming pathogens in other regions of the world.

Nematode management has previously been based to a great extent on chemical control, particularly in industrialized countries.

However, because of environmental and human safety concerns, many nematicides have been withdrawn from the market. The potential for nematicides to contaminate water, as well as the cost of applying chemicals, has increased the urgency to seek safer and more economical chemicals and to develop other means of management.

Resistant cultivars can be the best and most economical means of managing nematodes. Several commercial cultivars of peanut with resistance to *Meloidogyne* spp. are available in the USA, which has reduced grower dependence on nematicides. Peanut cultivars with resistance to *T. brevilineatus* are also available in India, and cultivars with resistance to *D. africanus* are

under development. Landraces of Bambara groundnut with resistance to *Meloidogyne* spp. have also been identified. Expanded utilization of cultural practices such as crop rotations, cover crops, trap crops, fallowing and flooding, organic amendments and other tactics that aid in reducing nematode damage is necessary for the maintenance of economical peanut production. More research is needed on understanding and predicting naturally suppressive soils, so as to aid producers in capitalizing on this important and greatly underutilized tactic of nematode management. Efforts to prevent the spread of nematodes through sanitation and quarantine in extreme situations may contribute to future containment of nematode problems.

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12 Nematode Parasites of Citrus*

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Citrus is grown in more than 140 countries in a belt within the 35° latitude north or south of the equator with Brazil, followed by China and the USA, being the largest producers. Within the family Rutaceae, the genera *Citrus* – oranges (*Citrus sinensis*), mandarins (*Citrus reticulata*), pomelos (*Citrus maxima*), grapefruit (*Citrus paradisi*), lemons (*Citrus limon*), limes (*Citrus aurantifolia*) and citrons (*Citrus medica*) – *Fortunella* (kumquats, *Fortunella japonica* and *Poncirus* (trifoliate oranges, *Poncirus trifoliata*) contain the principal commercial species (Swingle and Reese, 1967). Citrus production worldwide exceeded 121 Mt in 2013/14 (FAO, 2016). Approximately 62% of the world's citrus production is consumed as fresh fruit, while about 38% of the total production is processed for international trade (USDA, 2016). Oranges (53%), mandarins and tangerines (21%), lemons and limes (11%), grapefruit and pomelos (6%) are the citrus crops with the highest production tonnage (Cimen and Yesiloglu, 2016).

Citrus spp. are naturally deep-rooted plants (Ford, 1954) and optimum growth requires deep, well-drained soils because roots will not grow into or remain in saturated zones. Nevertheless, trees can be well managed in areas with high water tables if grown on beds. A tendency has developed in the USA and elsewhere to

increase early returns by planting higher-density, intensively managed orchards with shorter life expectancies due to such diseases as citrus blight, tristeza and, especially, huanglongbing (citrus greening) (Hearn, 1986; Campos-Herrera *et al.*, 2014). The most commonly encountered biotic stresses on citrus include Citrus tristeza virus, *Phytophthora* and parasitism by the citrus nematode *Tylenchulus semipenetrans* (Soost and Cameron, 1975).

Citrus Nematodes

The first association between nematode pests and citrus was observed by Neal (1889), and numerous nematode species have since been associated with citrus roots and their surrounding rhizospheres (Cohn, 1972; Duncan, 1999; Nehru *et al.*, 2005; Siddiqui, 2005; Park *et al.*, 2009b; Pathak and Chandra, 2010; Bakr *et al.*, 2011; Zalpuri *et al.*, 2013; Ibrahim *et al.*, 2016). Few of these plant parasitic nematode species, however, have been shown to be of economic importance to citrus crops. With the notable exception of *T. semipenetrans*, most nematode species capable of damaging mature citrus tend to be regional or local problems, due either to edaphic

*A revision of the chapter by L.W. Duncan in the second edition.

conditions or to the natural distribution of a particular nematode (Duncan, 1999; Etebu and Nwauzoma, 2014).

Tylenchulus semipenetrans

The 'citrus nematode' *T. semipenetrans* is aptly named, since it occurs in all citrus-producing regions of the world and limits the production of citrus fruit under a wide range of environmental and edaphic conditions. In the major citrus-producing regions, various surveys estimated that the nematode infested from 24–60% (Florida and California) to as many as 70–90% (Brazil, Spain, Texas and Arizona) of commercial orchards. Similar statistics are reported worldwide (Van Gundy and Meagher, 1977; Heald and O'Bannon, 1987; Esser *et al.*, 1993; Sorribas *et al.*, 2000; de Campos *et al.*, 2002; Iqbal *et al.*, 2006; Mukhtar *et al.*, 2007; Javed *et al.*, 2008; Safdar *et al.*, 2010; Emre and Kaskavalc, 2015). The expansion of citrus into new areas presents an important opportunity to reduce the incidence of *T. semipenetrans*. For example, the incidence of the nematode declined in Florida when orchards were relocated southward to avoid freeze damage to trees. New orchards were planted in non-infested soil, with trees certified free of the nematode. The widespread use of nematode-resistant rootstocks in older orchards also reduced the economic importance of this nematode in Florida (Duncan *et al.*, 1994b).

T. semipenetrans was first detected on citrus roots in California in 1912, and named and described during the next 2 years (Cobb, 1913, 1914). The nematode causes the disease 'slow decline' of citrus. The primary effect of *T. semipenetrans* in newly infested sites is a gradual reduction in tree quality, so that over a period of years infected trees are smaller, less vigorous and less productive than normal. The name 'slow decline' is less appropriate when young trees are replanted into heavily infested soil, where effects on tree growth may be noted soon after planting.

Molecular analyses revealed that *T. semipenetrans* form a monophyletic group (Tanha Maafi *et al.*, 2012; Rashidifard *et al.*, 2015a,b). Analysis of genes other than 28S ribosomal DNA (rDNA) is needed for further molecular resolution of biotypes of the citrus nematode.

Symptoms

Citrus nematodes do not kill the citrus trees they infect (O'Bannon and Esser, 1985). However, where citrus nematodes occur in high population densities, gradual decline of infected trees occurs to various degrees (Kwaye *et al.*, 2008). Symptom development depends on overall orchard conditions. Infected trees growing under otherwise optimum conditions may yield somewhat less fruit while appearing quite healthy. As conditions become less suitable for tree growth, the effects of citrus nematode parasitism are more apparent (Van Gundy and Martin, 1961; Van Gundy *et al.*, 1964; Heald and O'Bannon, 1987). In new citrus plantings, symptom development progresses slowly as nematode densities develop to high levels (Cohn, 1965b). The symptoms of *T. semipenetrans* parasitism are those associated with poor root development (Etebu and Nwauzoma, 2014). Leaves are smaller and may become chlorotic. In saline conditions, excessive sodium accumulates in leaves (Mashela and Nthangeni, 2002), and 'slow decline' symptoms are more apparent (O'Bannon and Esser, 1985; Duncan *et al.*, 1995; Levy and Syvertsen, 2004). Wilting occurs earlier during periods of water stress, and leaf drop is more pronounced, producing exposed branch terminals.

Heavily infected feeder roots are slightly thicker than healthy roots, and have a dirty appearance due to soil particles that adhere to gelatinous egg masses on the root surface (Fig. 12.1). Symptoms may not be apparent on lightly infected root systems, so that infected nursery stock may easily go undetected. Feeder roots decay faster due to loss of integrity at the epidermis and at feeding sites in the cortex, resulting in invasion by secondary organisms (Schneider and Baines, 1964; Cohn, 1965b; Hamid *et al.*, 1988). This may be expressed as lesions on lightly infected roots, while heavy infections result in cortical sloughing and root death. Parasitism results in increased levels of the cell-damaging enzymes superoxide dismutase, peroxidase and others (Abd-Elgawad *et al.*, 2014; Afify *et al.*, 2014).

Biology and ecology

The biology of *T. semipenetrans* is described in Chapter 2, this volume. The life cycle is regulated

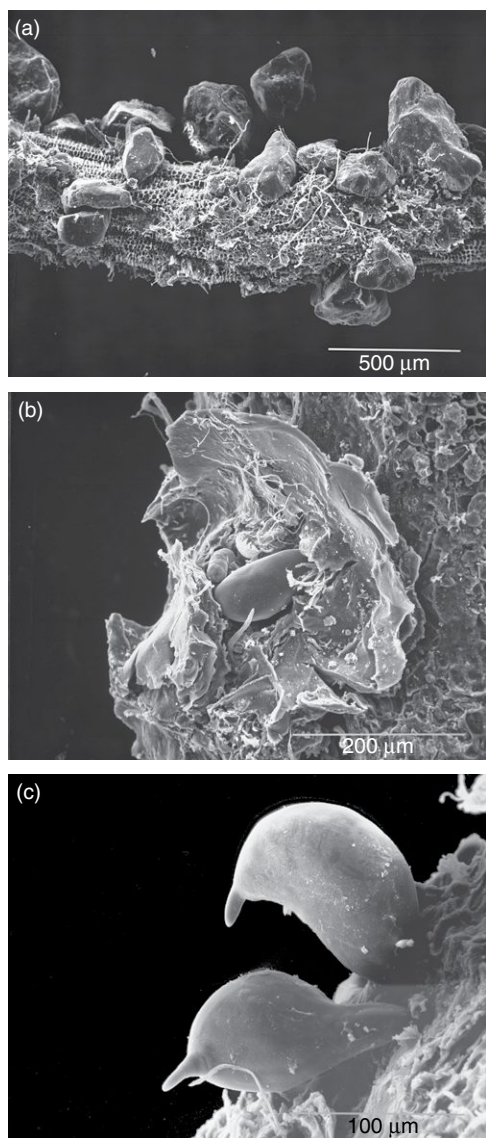


Fig. 12.1. Scanning electron micrographs of *Tylenchulus semipenetrans* on citrus roots. Sand grains adhere to the gelatinous matrix of the egg mass, giving the roots a dirty appearance (a); when sand is removed gently, the gelatinous matrix (desiccated from the fixation process) is seen surrounding the female, eggs and hatched juveniles (b); removal of the egg mass reveals the posterior ends of two females (c). (Photograph courtesy of L. Duncan.)

by host phenology interacting with geographic variation and temporal changes in the soil environment. Most studies reported one (Prasad and

Chawla, 1966; Bello *et al.*, 1986; Sorribas *et al.*, 2000), two (Vilardebo, 1964; O'Bannon *et al.*, 1972; Salem, 1980; Baghel and Bhatti, 1982; Duncan *et al.*, 1993; Al Hinai and Mani, 1998; Sorribas *et al.*, 2000; Galeano, 2002) or three (Hamid *et al.*, 1988) distinct periods of active population growth per year, although no seasonality was evident during a survey in Israel (Cohn, 1966). When conditions are otherwise favourable, populations will increase between temperatures of 20 and 31°C, with maximum development at 25°C and very slow development at the extremes (O'Bannon *et al.*, 1966). Summer soil temperatures in places such as Egypt, Jordan, Texas, Oman and Spain approach the upper limit of this range, and often correspond to population decline of the nematode (Salem, 1980; Davis, 1984; Al Hinai and Mani, 1998; Sorribas *et al.*, 2000; Al-Azzeh and Gharbieh, 2005). Similarly, in Arizona and Florida, population growth was slow on young trees until the canopies developed sufficiently to shade the soil and provide optimum soil temperatures (Reynolds and O'Bannon, 1963a).

Soil moisture is often inversely related to population growth of *T. semipenetrans* (Duncan *et al.*, 1993; Sorribas *et al.*, 2000; Galeano, 2002; Salahi Ardakani *et al.*, 2014), even though, compared with many plant parasitic nematodes, *T. semipenetrans* has little capacity for anhydrobiotic survival, and nematode densities decline quickly when trees become drought stressed (Van Gundy and Martin, 1961; Van Gundy *et al.*, 1964; Tsai and Van Gundy, 1988). Nevertheless, population densities in extremely dry parts of the rhizosphere can either grow rapidly or decline precipitously, depending on whether part or all of the root system is affected by drought (Fig. 12.2). Hydraulic lift of water deep in the soil to drier surface soil horizons via the root xylem (Caldwell *et al.*, 1991) creates an environment highly favourable for population growth of *T. semipenetrans* (Duncan and El-Morshedy, 1996). It is not known whether this is due to increased oxygen (Van Gundy *et al.*, 1962), passive movement of nematodes deeper in soil with precipitation, increased activity of natural enemies, or other factors (Sorribas *et al.*, 2000). *T. semipenetrans* may have experienced less selection pressure for anhydrobiotic survival through co-evolution exclusively with deep-rooted woody perennials. The potential importance of hydraulic

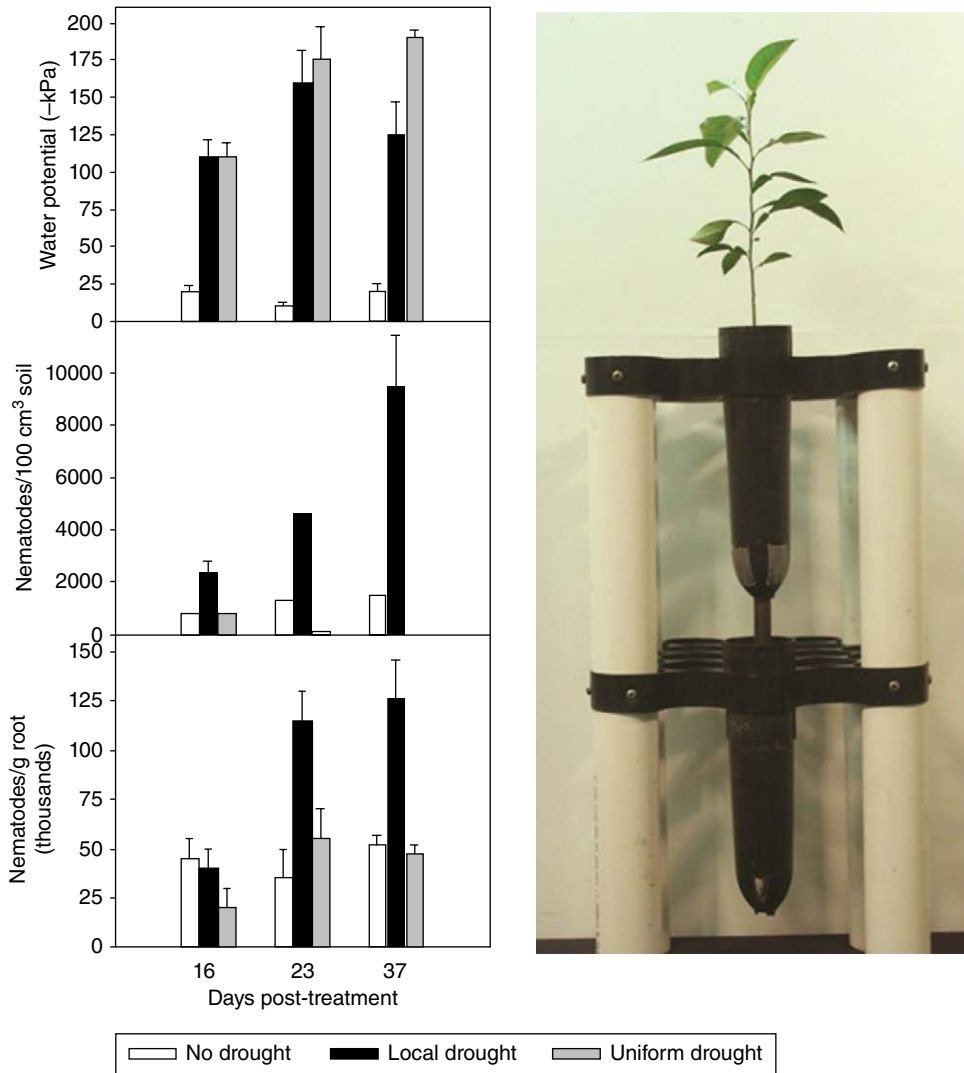


Fig. 12.2. The effect of soil water potential and hydraulic lift on population growth of *Tylenchulus semipenetrans*. Citrus seedlings were grown in double vertical pots in which the top pot was infested with nematodes. Three treatments consisted of irrigating both pots (no drought), only the bottom pot (local drought), or neither pot (uniform drought). Hydraulic lift of water from the lower to the upper pot could occur only under the local drought treatment. Despite similar soil water potential in the upper pot (top panel) under uniform and local drought, nematode population growth (lower two panels) was favoured by dry soil combined with hydraulic lift. (Redrawn from Duncan and El-Morshedy, 1996.)

lift for *T. semipenetrans* is consistent with reports that peak densities of this nematode in subtropical regions tend to be bimodal, occurring in the dry months that precede and follow the summer rainy season (Toung, 1963; Prasad and Chawla, 1966; O'Bannon *et al.*, 1972) and the observation in Mediterranean climates that

higher densities tend to occur under drip, compared with flood, irrigation (Sorribas *et al.*, 2000).

The phenology of citrus growth and development also affects the population growth of *T. semipenetrans*. When soil temperature and moisture levels are not limiting, fibrous root growth alternates with the growth of new

leaves. Flushes of new fibrous roots permit increased population growth of citrus nematodes in young roots that are most suitable for penetration and development of these nematodes (Cohn, 1964; O'Bannon *et al.*, 1972). In California (USA), three annual flushes of root growth corresponded to three distinct peaks in densities of *T. semipenetrans* females (Hamid *et al.*, 1988). The effect of the nematode on the normal pattern of root growth was demonstrated by reducing their densities with application of the nematicide oxamyl. Trees infected heavily by citrus nematodes initiated 66% more new roots, but the root mass was reduced by 30% due to the demand of the nematode for carbohydrates (Hamid *et al.*, 1988). The amount of carbohydrates available to nematodes is seasonal, and decreases markedly during the summer. Starch is an important nutrient for *T. semipenetrans* (Cohn, 1965a; Fig. 12.3), and the concentrations of starch and some sugars in fibrous roots are highly correlated with peaks in seasonal densities of this nematode pest (Duncan and Eissenstat, 1993; Duncan *et al.*, 1993). The concentrations of phenolic and lignin secondary compounds in citrus roots also vary seasonally and have been shown to be related inversely to *T. semipenetrans* population growth (Van Gundy and Kirkpatrick, 1964; Duncan *et al.*, 1993).

T. semipenetrans is broadly adapted to most edaphic conditions common to citriculture. The nematode will survive in any soil of which the

texture is suitable for citrus; although, unlike many nematode parasites, development in pot studies is often less rapid in sandy soils. Moderate amounts of clay and silt (Van Gundy *et al.*, 1964; Davide, 1971; Bello *et al.*, 1986; Salahi Ardakani *et al.*, 2014) and organic matter (O'Bannon, 1968; Salahi Ardakani *et al.*, 2014) favour infection and development. Populations develop best at elevated pH; however, at less optimum pH, the nematode is also pathogenic to citrus (Van Gundy and Martin, 1962; Bello *et al.*, 1986; El-Borai *et al.*, 2003a; Javed *et al.*, 2008; Salahi Ardakani *et al.*, 2014). Densities of citrus nematodes in Spain and Iran were related inversely to soil nitrogen (N) but favoured by soil potassium (K) (Sorribas *et al.*, 2008; Salahi Ardakani *et al.*, 2014). Although *T. semipenetrans* population growth is not favoured by saline soil solutions (Kirkpatrick and Van Gundy, 1966), population density and damage to trees are often very high in orchards irrigated with saline water (Machmer, 1958; Cohn *et al.*, 1965). Mashela *et al.* (1992a,b) demonstrated that both resistant and susceptible citrus seedlings exposed temporarily to salinity and then grown under non-saline conditions were predisposed to higher nematode reproduction and suffered greater nematode damage than seedlings grown without prior exposure to salinity. Similar conditions occur during the rainy season in orchards irrigated with salinized water during the dry season. Increased arginine synthesis leading to a

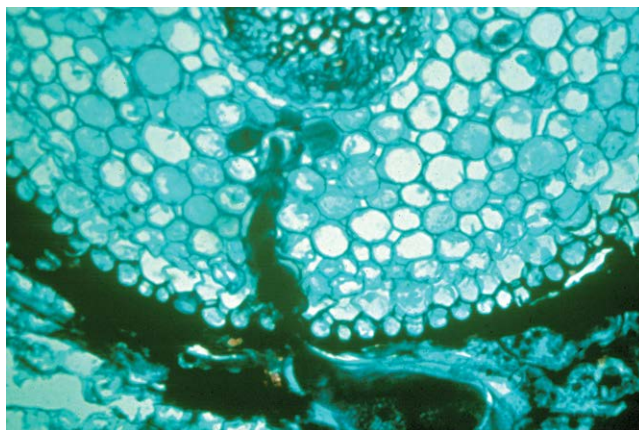


Fig. 12.3. Cross section of a feeder root showing extension of the *Tylenchulus semipenetrans* female body into the root cortex and densely stained nurse cells surrounding the head. (Photograph courtesy of R. Inserra.)

reduction in phenylalanine ammonia lyase was demonstrated in salt-stressed citrus plants, and might result in fewer phenolic compounds for defence against nematodes (Dunn *et al.*, 1998). *T. semipenetrans*-infected trees accumulated higher concentrations of Na and Cl in leaves, reduced concentrations in roots, and experienced greater nutrient deficiencies (particularly K) in both leaves and roots than did non-infected trees under salinity (Van Gundy and Martin, 1961; Milne and Willers, 1979; Mashela and Nthangeni, 2002). A demonstrated increased rate of carbon flow to nematode-infected roots is consistent with an osmotic-based mechanism proposed to explain the variable effect of the nematode on concentrations of different elements in roots and leaves (Mashela and Nthangeni, 2002).

Reproductive rates of different biotypes of the nematode obviously vary with rootstock (O'Bannon and Hutchinson, 1974). Even on susceptible commercial rootstocks, reproduction rates may differ considerably (Davide, 1971; O'Bannon *et al.*, 1972). While the scion is reported not to influence resistance or susceptibility of a rootstock, it does influence the general quality of the root system in terms of nematode development (Kirkpatrick and Van Gundy, 1966; Bello *et al.*, 1986). Nematode morphology is also affected to some degree by the host species of citrus (Das and Mukhopadhyaya, 1985). Tree nutrition influences population densities (Martin and Van Gundy, 1963; Mangat and Sharma, 1981).

Biotypes and rootstock resistance

In general, the citrus nematode has a narrow range of host genera. Although 75 rutaceous species (mainly citrus and citrus hybrids) support the nematode, only a few non-rutaceous hosts have been identified, the most important of which are grape (*Vitis vinifera*), olive (*Olea europaea*) and persimmon (*Diospyros virginiana*) (Kwaye *et al.*, 2008; Mashela *et al.*, 2012; Mathabatha *et al.*, 2015). Rashidifard *et al.* (2015a,b) reported pomegranate (*Punica granatum*) as a new host of *T. semipenetrans*.

Physiological races or biotypes of *T. semipenetrans* exist based on host suitability (Baines *et al.*, 1969a,b). Three biotypes are commonly recognized (Inserra *et al.*, 1980, 2009; Gottlieb

et al., 1986; Verdejo-Lucas *et al.*, 1997). A 'Citrus' biotype was described from populations found throughout the US citrus-growing regions and Italy. It reproduces poorly on *P. trifoliata* but will reproduce on *Citrus* spp. and on the hybrids 'Carrizo' and 'Troyer' citrange, as well as on olive, grape and persimmon. The 'Poncirus' biotype reproduces on most citrus, including *P. trifoliata*, and on grape, but not olive. It is reported from the USA, South Africa, Japan and Spain (Murguía *et al.*, 2005; Kwaye *et al.*, 2008; Mashela *et al.*, 2012; Mathabatha *et al.*, 2015). A 'Mediterranean' biotype is similar to the 'Citrus' biotype, except that it does not reproduce on olive (Stokes, 1969; Inserra *et al.*, 1980, 2009; Mathabatha *et al.*, 2015). It is found throughout the Mediterranean region, including Spain, Italy, Egypt, Turkey, Morocco, Israel, Algeria, Tunisia, Cyprus and Jordan, and perhaps India. Populations of a reported 'Grass' biotype that does not infect citrus have since been assigned to the species *Tylenchulus graminis* and *Tylenchulus palustris* (Stokes, 1969; Inserra *et al.*, 1988).

More recently, the genus has been investigated based on rDNA sequence (Van Megen *et al.*, 2009; Tanha Maafi *et al.*, 2012; Rashidifard *et al.*, 2015a,b) and PCR-ITS-RFLP (internal transcribed spacer-restriction fragment length polymorphism) profiles (Wang *et al.*, 2004; Park *et al.*, 2009a; Tanha Maafi *et al.*, 2012). Liu *et al.* (2011) designed specific primers for *T. semipenetrans* from China. In addition, a loop-mediated isothermal amplification (LAMP) assay has been developed that seems to be highly specific for the detection of different geographical populations of *T. semipenetrans* (Lin *et al.*, 2016).

Since the biotypes vary by geographic region, so do suitably resistant cultivars. Within citrus, several cultivars of *P. trifoliata* are resistant to most populations of *T. semipenetrans* (Inserra *et al.*, 1988; Verdejo-Lucas and Kaplan, 2002; Kwaye *et al.*, 2008; Legua *et al.*, 2011; Mashela *et al.*, 2012; Mathabatha *et al.*, 2015). Resistant hybrids of *P. trifoliata* also provide acceptable rootstocks in some regions (Gottlieb *et al.*, 1986; Spiegel-Roy *et al.*, 1988; Verdejo-Lucas *et al.*, 2000; Javed *et al.*, 2008; Uzun *et al.*, 2011; Ibáñez, 2013). Swingle citrumelo (*C. paradisi* × *P. trifoliata*) is a commercially acceptable rootstock with a high degree of resistance to

most populations of *T. semipenetrens*. It is also resistant to tristeza virus and tolerant of *Phytophthora nicotianae*, and is widely planted in Florida; however, it is intolerant of calcareous soils. In Iran, *P. trifoliata*, Swingle citrumelo and *Citrus aurantium* are recommended for managing the citrus nematode (Ayazpour *et al.*, 2008). Several hybrids of *P. trifoliata* × various mandarin (*C. reticulata*) rootstocks have inherited high resistance to *T. semipenetrens*, grow well in calcareous soils and are being evaluated for use in Spain (Verdejo-Lucas *et al.*, 2003; Legua *et al.*, 2011). Selections of Poorman orange (*Citrus* × hybrid of undetermined origin) × *P. trifoliata* hybrids exhibiting combined resistance to *Phytophthora citrophthora* and tristeza were found to be highly resistant to more than one biotype of the nematode (Gottlieb *et al.*, 1986; Spiegel-Roy *et al.*, 1988). Carrizo citrange, Savage citrange and Yuma citrange (Iqbal *et al.*, 2005), citrumelo and grapefruit (Javed *et al.*, 2008) were reported as resistant to the citrus nematode in Pakistan. In Western Australia, Troyer and Carrizo citrange are used as tolerant to the citrus nematode, *Phytophthora* and Citrus tristeza virus (Lacey and Foord, 2006). Louzada *et al.* (2008) indicated that the cultivars C22, C57 and C146 were tolerant to the citrus nematode in Texas (USA).

Factors identified as responsible for the resistance of citrus to *T. semipenetrens* development include host cell hypersensitivity, wound periderm formation, compounds in root tissues which are toxic to the nematode, and allelopathy or unidentified factors, which result in low rhizoplane nematode densities early during the infection process, decreased female fecundity and a higher proportion of males (Van Gundy and Kirkpatrick, 1964; Kaplan and O'Bannon, 1981; Verdejo-Lucas *et al.*, 2000; Galeano *et al.*, 2003; Kallel *et al.*, 2006). Resistance inherited from *P. trifoliata* is thought to be a dominant and oligogenic trait (Hutchinson, 1985). Eleven random amplified polymorphic DNA (RAPD) markers associated with resistance can facilitate the identification of resistance in breeding programmes (Ling *et al.*, 2000). Xiang *et al.* (2010) used marker-assisted analysis to show that some of the markers were efficient for detecting both citrus nematode resistance on the gene locus '*Tyr1*' and the Citrus tristeza virus resistance gene locus '*Ctv*'.

Disease interactions

T. semipenetrens can increase the virulence of *Fusarium solani* (O'Bannon *et al.*, 1967; Labuschagne *et al.*, 1989; Walker and Morey, 1999). *P. nicotianae* is a more virulent pathogen of citrus roots than *F. solani*, and frequently occurs in combination with *T. semipenetrens*. Levels of *P. nicotianae* in soil increased in a field trial when *T. semipenetrens* were controlled with nematicides (Graham and Duncan, 1997). Subsequently, it was shown that pre-infection of citrus roots by *T. semipenetrens* could reduce the rate of infection by *P. nicotianae* (El-Borai *et al.*, 2003b). *Fusarium semitectum* and *T. semipenetrens* were found at high levels in Pakistan orchards, and damage to seedlings infected with both pathogens was synergistic (Safdar *et al.*, 2013).

Economic importance and damage thresholds

Although *T. semipenetrens* influences citrus yields differently under various circumstances, guidelines have been published to help interpret soil sample results. It was estimated in California that soil stages (juveniles/100 g soil) below 800 represented a non-damaging level (Garabedian *et al.*, 1984). Orchards with densities greater than 1600 may respond economically to nematicide treatment, and above 3600 treatments may improve yield substantially. Densities were estimated during the peak growth period of May–July. Females per g root are also used in California to define damage levels, with counts of less than 300, more than 700 and more than 1400 representing low, moderate and high ranges, respectively. The threshold was approximately 850 juveniles/100 cm³ soil when densities were measured during periods of low population development. Nevertheless, loss estimates for citrus nematode derived from Seinhorst's yield loss model and other functions vary extensively between systems (Timmer and Davis, 1982; Sorribas *et al.*, 2008; Abd-Elgawad *et al.*, 2016).

When citrus is sold on the fresh fruit market, larger-size fruit obtain premium prices. Because *T. semipenetrens* often reduces fruit size, the nematode can be of greater economic importance in orchards where the fruit is marketed fresh rather than for processing (Philis, 1989; McClure and Schmitt, 1996).

Methods of diagnosis

SAMPLING AND EXTRACTION: because nematodes are aggregated in soil and along roots, sample size can be reduced by sampling during seasons of peak population size and in locations of highest feeder root and nematode concentration (Nigh, 1981a; Duncan, 1986). The stratification of orchards into areas of healthy and unhealthy trees may also improve sample precision (Scotto la Massèse, 1980). Seasonal variation in soil and root nematode densities are in the order of three- to tenfold (Salem, 1980; Baghel and Bhatti, 1982; Duncan *et al.*, 1993; Sorribas *et al.*, 2000). Thus, for comparative purposes, it is helpful to sample during the same season each year, preferably when peak densities are present. Similarly, feeder roots and nematodes are more abundant beneath the tree canopy than at the dripline or in rows between trees (Nigh, 1981b; Davis, 1985; Duncan, 1986). Low-volume irrigation systems concentrate root and nematode densities even further in the wetted zones.

Most published work on sample size indicates that accurate estimation of *T. semipene-trans* density is costly, requiring from 30 to 75 soil cores in 2 ha to estimate population levels within 20–40% of the true mean (McSorley and Parrado, 1982a,b; Davis, 1984; Duncan *et al.*, 1989, 1994a). Despite its low precision, sampling is valuable since the majority of density estimates are well above or below management threshold levels. Some laboratories suggest that samples be obtained to a depth of at least 60 cm; although, in a study conducted in a shallow-rooted citrus orchard, the density levels in the top 30 cm of soil reflected the population level to 60 cm depth (Duncan, 1986). Fibrous root mass density and the density of root stages of the nematode can also be obtained from soil samples. For a given sample size, sample precision for root stages of the nematode is less than that for soil stages (Duncan *et al.*, 1993).

Laboratories frequently determine infestation levels as nematodes per unit soil weight or volume. Juveniles and males of *T. semipenetrans* can be separated from soil by most conventional methods. Extraction efficiencies are rarely reported, and it is often difficult to make direct comparisons between laboratories. For some soils, techniques based on Baermann funnels appear to be similar in efficiency to techniques

employing density flotation if the layer of soil extracted is relatively thin (Nigh, 1981b; Rashidifard *et al.*, 2015a,b). However, other authors report major differences in the efficiency of the two approaches (Galeano, 2002). A disadvantage to quantifying soil stages is that a given population level may represent a different parasitic burden depending on the root mass density of the tree (Scotto la Massèse, 1980; Duncan, 1986). Nematodes hatching from root samples are obtained easily (Young, 1954; Cohn *et al.*, 1965; McClure and Schmitt, 1996), and females per unit root can also be determined by extraction (Baines *et al.*, 1969b; Duncan *et al.*, 1993) or direct counts on stained roots (Davis and Wilhite, 1985).

ESTIMATING CROP LOSS: economic loss assessment in mature perennial crops is complicated by the fact that the difference in yields between nematode-infected and non-infected trees is due to long-term, cumulative stress. The nematodes on the root system affect the current crop; however, infected trees may also be smaller and less healthy, due to previous effects of parasitism. As trees decline, they tend to support fewer nematodes. Other soil-borne factors frequently contribute to tree decline in addition to nematodes, such as *Phytophthora* spp., salinity, poor soil drainage, drought and nutrient deficiency. Moreover, if stresses are removed, citrus trees allocate carbohydrate to vegetative growth before fruit growth (Eissenstat and Duncan, 1992). Thus, yields may (McClure and Schmitt, 1996) or may not increase in the first year following nematode management (Duncan, 1989; Le Roux *et al.*, 1991).

Two approaches have been widely employed for citrus nematode crop loss assessment: (i) nematode population densities have been reduced with nematicides and subsequent yields monitored; and (ii) the relationship between nematode infestations and yields has been examined. Both approaches have limitations. It is evident from the bulk of reported evidence that the citrus nematode can reduce tree health and fruit yield and quality, but it is often not clear to what extent other factors may have influenced the results of these studies. When orchards are treated with nematicides, rhizosphere organisms in addition to nematodes are affected (Baines *et al.*, 1962, 1966; Mankau, 1968; Milne and du Toit, 1976; O'Bannon and Neme-

1978). In the case of systemic chemicals, above-ground pests and other fauna associated with the tree may also be affected (Milne and De Villiers, 1977; Childers *et al.*, 1987). Chemical treatments may also directly affect plant development negatively (Cohn *et al.*, 1968; Timmer, 1977) or positively (Wheaton *et al.*, 1985). Similarly, relating crop yields to nematode infestation levels can be confounded by unmeasured edaphic variables that affect both nematode and tree. There are no reports of experiments comparing the growth and yield of citrus trees in the field that are randomly inoculated with *T. semipenetrans*. Such experiments would provide important information and are feasible because the nematode moves very slowly in an orchard if unaided by flowing water or other cultural practices (Duncan *et al.*, 1995).

Experiments in which nematicide treatments have resulted in significant citrus yield increases have been widely reported (Baines, 1964; Yokoo, 1964; Cohn *et al.*, 1965; Oteifa *et al.*, 1965; Philis, 1969; O'Bannon and Tarjan, 1973; Vilardebo *et al.*, 1975; Davide and Dela Rose, 1976; Milne and Willers, 1979; Timmer and Davis, 1982; Childers *et al.*, 1987; Duncan, 1989; Le Roux *et al.*, 1991, 1998; Tanha Maafi and Dmadzadeh, 2008; Deepa *et al.*, 2011; Hammam *et al.*, 2016). Treatment responses in these and other experiments ranged from none to several hundred per cent increase in fruit from treated trees. Although yield response to nematicide treatment is often positive, results have been erratic (e.g., Davis *et al.*, 1982; Davis and Wilhite, 1985; Stirling and Wachtel, 1985).

Studies relating tree quality and yield to nematode infestation level report similar findings. Under uniform soil conditions within orchards (Reynolds and O'Bannon, 1963b; Scotto la Massèse, 1980; Coelho *et al.*, 1983), or considering specific cultivars between orchards (Davide, 1971), the highest levels of soil stages of *T. semipenetrans* frequently were measured beneath trees with only moderate symptoms. Healthy trees supported lower densities that had not yet caused significant damage, while the reduced root systems of severe decline trees were incapable of supporting high nematode densities. In Israel, the average tree quality index declined with nematode infection level beyond a specific threshold level (40,000 nematodes/g root) (Cohn *et al.*, 1965). Citrus fruit yield has

also been correlated negatively with infestation level (Willers, 1979; Timmer and Davis, 1982; Childers *et al.*, 1987; Sorribas *et al.*, 2008).

A Florida orchard was identified in which randomly distributed trees were or were not infected by *T. semipenetrans*. Average soil texture, levels of salinity and nutrients, density of *P. nictianae* and tree decline symptoms did not differ for infected or non-infected trees. However, leaf area, fibrous root mass density and fruit yield of infected trees were 32, 8 and 22% lower, respectively, than those from non-infected trees (Duncan *et al.*, 1995).

Management

Methods commonly employed to control *T. semipenetrans* depend on local conditions, and focus on: (i) excluding the pest; (ii) minimizing losses through crop management; and (iii) reducing population densities of the pest.

EXCLUSION: most citrus-growing regions have few serious nematode pests, so that the exclusion of *T. semipenetrans* from orchards is a realistic goal to preclude the perennial expense of nematode management. Occasional introductions of *T. semipenetrans* into non-infested orchards do not negate the value of a conscientious sanitation programme, since the nematode migrates very slowly by its own power (Meagher, 1967; Tarjan, 1971; Baines, 1974; Duncan *et al.*, 1995). In the absence of flooding, and particularly with the use of low-volume irrigation, trees may remain uninfected for long periods, despite the existence of nematodes on adjacent trees. Since the host range of the nematode is limited to only a few non-rutaceous plant species, infestation usually results from movement of infected planting stock (Van Gundy and Meagher, 1977) or from contaminated equipment (Tarjan, 1956). Programmes to approve and monitor nursery sites and certify that nursery stock is nematode free have been highly effective in limiting the distribution of *T. semipenetrans* (Milne, 1982; Lehman, 1995). The Florida nursery certification programme saved growers US\$33 million in 1994 by reducing yield losses from *T. semipenetrans* that otherwise would have occurred from the spread of this nematode (Lehman, 1995). Such programmes focus on: (i) continuous monitoring through soil sampling; (ii) isolating

nursery locations to avoid runoff water from infested orchards; and (iii) security to prevent contaminated planting media or equipment from entering the nursery area. Separate equipment for use in infested and non-infested orchards may be feasible in some cases; otherwise, equipment must be disinfested continually prior to movement into non-infested orchards (Esser, 1984). Irrigation with some forms of surface water, such as canals and rivers, has been found to represent a serious source of inter-orchard contamination by *T. semipenetrans* and *P. nicotianae* (Cohn, 1976), particularly since pests can be spread widely in a short time. Irrigation water can be decontaminated through the use of settling ponds and filtration systems, but the procedures require careful maintenance (Cohn, 1976).

CROP MANAGEMENT: a key concept for the successful management of *T. semipenetrans* is that of the limiting factor (Thomason and Caswell, 1987). Vigorous orchards in which nematode population densities exceed management thresholds are those in which nematode management is most likely to be profitable. Although citrus nematode may sometimes exacerbate damage caused by other stresses (Labuschagne and Kotze, 1988; Mashela and Nthangeni, 2002), citrus trees that are damaged by *Phytophthora* spp., poor drainage, salinity, frequent drought or other problems are unlikely to respond consistently to the management of *T. semipenetrans* only. Therefore, it is important to ensure that orchards are managed properly in all respects before investing in nematode management tactics.

DIRECT MANAGEMENT: the direct suppression of citrus nematodes relies on the use of resistant rootstocks, nematicidal chemicals, or physical methods such as solarization. The commercially acceptable resistant rootstock Swingle citrumelo is now planted widely in Florida and, combined with nursery certification, has reduced the occurrence of *T. semipenetrans* appreciably (Lehman, 1995). Resistance management appears to be an important consideration, because the Poncirus biotype occurs in regions with widespread use of *P. trifoliata* rootstocks (Baines *et al.*, 1969b). Resistance-breaking biotypes were detected on Swingle citrumelo in a Florida

nursery (Duncan *et al.*, 1994b). When resistant rootstocks are used to replant an entire orchard, they are challenged only by nematodes that remain from the previous trees. However, if resistant rootstocks are used to replace unthrifty individual trees in orchards with susceptible rootstocks, the opportunity to break resistance increases, because the resistant rootstock is challenged continuously by nematodes supported by the adjacent susceptible rootstocks (Duncan *et al.*, 1994b; Verdejo-Lucas *et al.*, 2003).

The most effective pre-plant nematicides in citrus are fumigants, such as metam sodium and 1,3-dichloropropene. The fumigants act directly on nematodes as contact poisons. Pre-plant fumigation of old orchard sites with histories of citrus nematode infestation can be important to prevent the rapid infection of young trees (Baines *et al.*, 1956, 1966; O'Bannon and Tarran, 1973; Le Roux *et al.*, 1998). Citrus nematodes are well adapted to survive in the absence of plants (Cohn, 1966; Van Gundy *et al.*, 1967), and have been detected in fields for as long as 9 years after the removal of citrus (Baines *et al.*, 1962; Hannon, 1964). Sorribas *et al.* (2003) demonstrated that trees on resistant rootstocks grew more quickly than on susceptible rootstocks in non-fumigated soil infested by *T. semipenetrans*, but not in soil fumigated with 1,3-dichloropropene. Fumigants can also affect young tree growth adversely under some conditions (Cohn *et al.*, 1968; Milne, 1974). It is important to observe proper intervals between treatment and planting to avoid phytotoxicity. In nurseries that experience frequent or very thorough fumigation, mycorrhizal fungi may nearly be eradicated (O'Bannon and Nemec, 1978). To avoid phosphorus deficiency, replanted nursery stock should be mycorrhizal, or seedbeds should be re-inoculated with endomycorrhizal fungi. This problem is seldom encountered when replanting orchards, since plants in fumigated sites are quickly invaded by fungi from adjacent soil if they are not mycorrhizal at the time of transplanting (Graham, 1988).

Pre-plant solarization of soil can also be highly beneficial to the subsequent growth of citrus, but the reasons why are unresolved. Increased tree growth and yield in response to solarization in South Africa are more likely to have resulted from early control of *P. nicotianae* than

from control of the citrus nematode (Cronjé *et al.*, 2002). Indeed, the increased growth of trees due to solarization resulted in generally higher densities of *T. semipenetrans* in solarized plots as long as 10 years following solarization.

Post-plant nematicides in citrus are generally carbamate or organophosphate, acetylcholinesterase inhibitors, but new chemistries with different modes of action are being registered (McKenry *et al.*, 2009; Faske and Hurd, 2015). Some post-plant citrus nematicides are translocated systemically within the tree, and suppress insects and mites (both pest and beneficial species) in addition to nematodes. Thus, like many pesticides, some of these nematicides have the potential to disrupt biological control in the canopy of the tree. All of the nematicides used in citrus are incorporated in the soil, either mechanically or with irrigation for efficacy and safety. They are inappropriate for small farms that lack proper safe application equipment. Several, such as aldicarb and fenamiphos, have been deregistered for use in citrus. The reported efficacy and profitability of these materials vary widely (Walker, 2007; Tanha Maafi and Damadzadeh, 2008; Deepa *et al.*, 2011; Eskander *et al.*, 2013; Amin and Youssef, 2014; Hammam *et al.*, 2016).

Nematicide placement, application timing and application history are important considerations. Nematicides in large commercial citrus orchards are applied in bands down the tree rows or through low-volume irrigation systems. Since the abundance of nematodes and feeder roots in the upper soil horizons declines quickly with distance from the trunk, nematicide bands, even for systemic products, are most effective when they are applied as much as possible beneath the tree canopy (Nigh, 1981a; Duncan, 1986, 1989). Applications through low-volume irrigation systems deliver nematicides to areas of highest root and nematode abundance. Treatment should precede periods when nematodes actively invade new roots (Hamid *et al.*, 1988). Splitting the maximum allowable nematicide dose for multiple applications within a season can increase efficacy markedly. The life cycle of the citrus nematode was disrupted by three applications of cadusaphos, made at 60-day intervals, to the extent that nematodes were not detected on roots or in the soil for up to 4 years (Le Roux, 1995; McClure and Schmitt, 1996; Antoon *et al.*, 2015). However, the profitability

of nematicide use cannot be assessed from studies of 2 or 3 years' duration, because continuous use can reduce the effectiveness of nematicides as a result of accelerated microbial degradation (Smelt *et al.*, 1996; Johnson, 1998). Moreover, under certain conditions of soil type, precipitation rate and water table level, the potential for groundwater contamination exists for most chemicals that are applied to the soil (Kaplan, 1988).

Biological control of *T. semipenetrans* remains largely a topic of research. There are several reports of the incidence of naturally occurring communities of nematophagous fungi and parasitic bacteria such as *Pasteuria* species in citrus orchards (Gaspard and Mankau, 1986; Fattah *et al.*, 1989; Sorribas *et al.*, 2000). Pathak *et al.* (2012, 2017) developed molecular probes and showed habitat variation in the prevalence of two of seven species of nematophagous fungi detected in the DNA extracted from samples of nematodes in citrus orchards. Ciancio *et al.* (2015) reported model parameters describing the dynamics of the predator–prey relationship between a *Pasteuria* sp. and *T. semipenetrans* in an Italian orchard as a basis to design augmentation programmes (Luc *et al.*, 2010). Predaceous mites prey on citrus nematodes in nature and regulate them under some controlled conditions (Walter and Kaplan, 1990; Walter *et al.*, 1993; Salehi *et al.*, 2014). A number of other biocontrol agents, such as *Pseudomonas fluorescens*, various *Trichoderma* spp., *Pochonia chlamydosporia* and *Purpureocillium lilacinum*, available commercially have been tested under controlled conditions and in the field, with variable results (Deka *et al.*, 2002; Walode *et al.*, 2008; Deepa *et al.*, 2011; Hammam *et al.*, 2016). Botanical extracts have been shown to affect citrus nematodes (Maile, 2013; Amin and Youssef, 2014; El-Zawahry *et al.*, 2014).

Additional nematode parasites of citrus

Nematodes other than *T. semipenetrans* currently known to damage citrus tend to be very limited in distribution. Both migratory endoparasites (lesion and burrowing nematodes) and sedentary endoparasites (root knot nematodes), as well as a number of species of ectoparasitic nematodes, can damage citrus.

Radopholus similis* and *Radopholus citri

Spreading decline is a severe disease of citrus caused by the citrus race of *Radopholus similis* that is only encountered on Florida's central ridge of deep sandy soils. *R. similis* is commonly called the burrowing nematode, because of its extensive tunnelling through root tissue. The disease was first described in 1928 and the causal organism was identified in 1953 (Suit and DuCharme, 1953). The name of the disease is descriptive of the rapid progression of decline, which can reach 15 m/year. The citrus race of *R. similis* also parasitizes banana, but is distinct from the more widespread banana race for which citrus is not a host (DuCharme and Birchfield, 1956). A coffee race that appears similar to the banana race has been described from Indonesia (Hulupi, 2006).

In 1984, the citrus race of *R. similis* was re-named *Radopholus citrophilus* and designated as a sibling species to *R. similis* based on putative differences in chromosome number, isozyme patterns, mating behaviour, host preference (Huettel *et al.*, 1984) and morphology (Huettel and Yaegashi, 1988). Subsequent research provided convincing evidence based on karyotype identity, morphological and genetic similarity and reproductive compatibility that *R. citrophilus* was a junior synonym of *R. similis* (Kaplan and Opperman, 1997, 2000; Kaplan *et al.*, 1997, 2000; Valette *et al.*, 1998). Genetic similarity among *R. similis* populations worldwide may result from its wide host range, combined with its recent dissemination worldwide on banana from its centre of origin somewhere in Australasia (Kaplan, 1994b; Fallas *et al.*, 1996; Machon and Bridge, 1996).

Radopholus citri was discovered in citrus roots in Indonesia (Bridge *et al.*, 1990; Hahn *et al.*, 1994; Machon and Bridge, 1996). The pathogenicity of *R. citri* has been demonstrated and the nematode is associated with declining trees in Indonesia.

Symptoms

Spreading decline is generally distinguishable from other major decline diseases, such as citrus blight, in that large contiguous groups of trees are affected and expansion of the diseased area is rapid. Forced water uptake in the trunk of the tree (Graham *et al.*, 1983) is indistinguishable from

normal trees, in contrast to trees suffering from citrus blight. Decline trees have sparse foliage, the leaves and fruit are small, and fewer mature fruit remain on the trees. Branch ends are bare, and eventually entire branches die. Affected trees wilt rapidly during periods of low soil moisture, particularly during the periods of drought that tend to occur in the winter and spring in Florida, when disease progression is most rapid.

Symptoms on roots are most apparent below 25–30 cm, so that evidence of damage to the abundant shallow portion of the root system may be lacking (Ford, 1952, 1953). The most obvious symptom in the root system is the reduction in the quantity of feeder roots in the deeper soil profiles. At depths of 25–50 cm, 75% of the root system may remain, but below this level the root system is almost totally destroyed. Since mature citrus growing on the deep sands of the ridge establish as much as half of the feeder roots between 1 and 6 m, destruction of the deep root system on a large tree accounts for the drought-related above-ground symptoms during periods of moisture stress. Nematodes may burrow in a section of root for several weeks, completely destroying the phloem and much of the cortex, girdling the central cylinder (DuCharme, 1959; Fig. 12.4). The nematode penetrates the region of elongation, and root tips can become swollen due to hyperplasia and stubby if terminals are penetrated (DuCharme, 1959, 1968).

Biology

R. similis on citrus has a life cycle of 18–20 days under optimum conditions (DuCharme and Price,

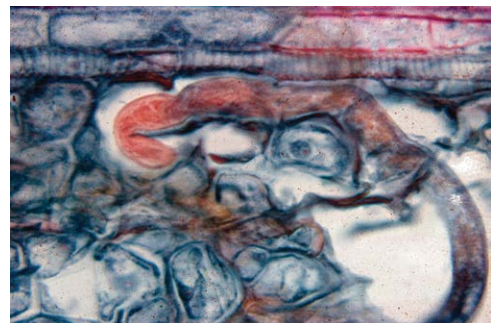


Fig. 12.4. Cavity created in fibrous root cortical tissue by *Radopholus similis* (note that the nematode does not penetrate the stellar tissues). (Photograph courtesy of J. O'Bannon.)

1966), permitting population densities to increase rapidly when conditions are favourable (DuCharme and Suit, 1967). Following root penetration, mature females begin to lay eggs at an average rate of nearly two per day, and eggs hatch in 2–3 days. In gnotobiotic culture, colonies initiated with single females attained an average of more than 11,000 individuals in less than 3 months, although rhizosphere competitors restricted population growth in orchards far below such a level (DuCharme and Price, 1966). The nematodes normally reproduce sexually; however, females that do not mate after time reproduce as hermaphrodites (Brooks and Perry, 1962; Kaplan and Opperman, 2000). Mature males do not feed, and comprise 0–40% of the population, averaging about 10% (DuCharme and Price, 1966). The nematode remains within the root until forced by overcrowding and decay to migrate.

Survival and means of dissemination

R. similis does not survive for long periods in the absence of host roots (DuCharme, 1955). In field trials in which root material was excluded, the nematode could not be detected in samples after 6 months (Tarjan, 1961). However, under more natural experimental conditions, the nematode has been detected after up to 14 months under bare fallow conditions (Hannon, 1963), and unconfirmed reports indicate as long as 2 years (Suit *et al.*, 1967). Large root fragments that remain buried in soil after tree removal may help support residual populations during fallow.

The nematode is spread in contaminated rootstock (Poucher *et al.*, 1967), machinery (Tarjan, 1956) and subsoil water (DuCharme, 1955), and it migrates rapidly along developing root systems. In orchards, the spreading decline disease is reported to move as much as 15 m/year (Poucher *et al.*, 1967), while in greenhouse tests, movement of about one-quarter to one-third of that rate has been measured (Feldmesser *et al.*, 1960; O'Bannon and Tomerlin, 1969a; Tarjan, 1971).

Host range

R. similis is remarkably polyphagous, attacking over 250 plants in 15 families outside of the Rutaceae (Ford *et al.*, 1960). Within the citrus and

closely related genera, more than 1200 species, cultivars and hybrids have been screened for resistance or tolerance to *R. similis* (Ford and Feder, 1961; O'Bannon and Ford, 1976). Three cultivars of citrus, Ridge Pineapple sweet orange, Estes rough lemon and Milam lemon, and a *P. trifoliata* × citrus hybrid, Carrizo citrange, have been released as rootstocks since 1958. Although data on tolerance under field conditions are very limited, all of the rootstocks have subsequently been shown to support biotypes of *R. similis* capable of breaking resistance (Kaplan and O'Bannon, 1985). In the case of Carrizo citrange, considerable variability exists within the progeny for susceptibility to burrowing nematodes (Kaplan, 1986); however, a breeding line known as Kuharski Carrizo has been identified in which resistance appears to be stable (Kaplan, 1994a).

Environmental factors affecting parasitism

The biology of *R. similis* related to citrus is influenced strongly by edaphic conditions. The nematode is found in citrus-growing regions of Florida other than the ridge, but do not develop to damaging levels. This is probably related to interactions between soil temperature, moisture and root growth periodicity. The cardinal temperature for *R. similis* is 24°C, and development occurs between 12 and 32°C. Optimum temperatures occur for the longest periods each year in the deeper soil horizons, where the highest level of reproduction is known to occur. Highest absolute soil densities are found in the late summer–early autumn period, when optimum temperatures combine with an annual cycle of root growth to support population increase. As the root growth cycle declines later in the autumn, infected roots begin to die and soil densities begin to decline, even though the nematodes recovered per unit of root tend to be highest in the late autumn (DuCharme, 1967, 1969). The temperature extremes in the surface soil horizon are nearer the limits for the development of *R. similis* during the period of root growth, which may partly explain low population development in surface roots. The nematode does not have a known resting stage, so that moisture deficits, which are more commonly encountered in the shallow horizons, may also inhibit development in this zone (Tarjan, 1961).

Soil texture is also an important determinant in the spreading decline disease cycle. The nematode is more pathogenic to citrus in pot studies in sandy than in loamy soils (O'Bannon and Tomerlin, 1971). Movement of *R. similis* is highest in light textured soil (Tarjan, 1971).

Disease complexes

Few reports exist of interactions between *R. similis* and other rhizosphere organisms (Feder and Feldmesser, 1961). Feldmesser *et al.* (1959) obtained indirect evidence that secondary fungal invaders played a key role in the disease complex when they treated infected seedlings with the fungicide captan, which increased nematode densities as well as the root and top weights of plants. Root lesions are infected quickly by fungi and other rhizosphere inhabitants (DuCharme, 1968). *R. similis* densities declined in the presence of mycorrhizal fungi, probably due to enhanced phosphorus uptake, because the effect was also obtained on plants growing with supplemental phosphorus (Smith and Kaplan, 1988). Similarly, citrus plant tolerance to *R. similis* appears to be enhanced by mycorrhizal infection when soils are deficient in phosphorus (O'Bannon and Tomerlin, 1971; O'Bannon and Nemec, 1979).

Biotypes

All burrowing nematode-resistant rootstocks support low densities of *R. similis*, and populations of *R. similis* have broken resistance in Milam lemon, Ridge Pineapple and Albritton sweet orange and Kuharski Carrizo citrange rootstocks (Kaplan and O'Bannon, 1985; Kaplan, 1994b).

Economic importance and damage threshold levels

R. similis and a lesion nematode, *Pratylenchus coffeae*, appear to be the most virulent nematode parasites of citrus worldwide (O'Bannon *et al.*, 1976). However, since *R. similis* distribution on citrus is restricted to Florida, the nematode's economic impact is slight on the world market. In 1972, it was estimated that *R. similis* caused 0.1–0.2% yield losses in the world citrus industry (Cohn, 1972). In infested orchards, the losses were estimated to be of the order of 40–70% for oranges and slightly higher for grapefruit (DuCharme, 1968). The damage by spreading

decline within orchards has been mitigated in recent years by the improved management practices described below. Unfortunately, the discontinuation of programmes to prevent the migration of burrowing nematodes from infested to uninfested orchards has increased the rate of spread of this pest.

Management

Management of spreading decline currently focuses on restricting the spread of the nematode through planting-stock certification, sanitation, orchard management, use of resistant rootstocks and use of nematicides. Based on the potential threat of spreading decline to citrus on Florida's ridge, planting stock must be certified as pest free. Nurseries are sampled regularly and inspected to remain certified. Commercial movement of soil within and into citrus-producing areas requires certification that the site of origin is pest free. A cost–benefit analysis of the value of the certification programme in reducing potential losses to burrowing nematode estimated a 14:1 return on investment, resulting in increased yield worth US\$40 million/year (Lehman, 1995). Equipment used in infested orchards should be reserved for that purpose when possible, or disinfested between operations (Esser, 1984).

The fact that *R. similis* damages primarily the deeper (below 45 cm) portion of the citrus root system provides the opportunity to manage spreading decline with cultural or management practices designed to support a healthy, shallow root system. Infested orchards in which sound practices are employed have remained economically viable (Tarjan and O'Bannon, 1977) and may out-produce annual state production averages (Bryan, 1969). Suggested practices include: the use of herbicides and mowing rather than cultivation, to avoid cutting surface roots (Tarjan and Simmons, 1966); the frequent use of supplemental irrigation (Bryan, 1969); and frequent fertigation to maintain nutrients in the shallow rhizosphere. Systemic nematicides such as oxamyl have been, and continue to be, used by some growers to reduce *R. similis* in deeper roots (O'Bannon and Tomerlin, 1977).

Three rootstocks are recommended for use against spreading decline, Milam lemon, Ridge Pineapple sweet orange and Kuharski Carrizo citrange. The occurrence of resistance-breaking

populations of the burrowing nematode indicates a need for rootstocks with additional resistance genes (Kaplan and O'Bannon, 1985). The discovery that *R. similis* is phylogenetically similar to cyst nematodes in the Haplaimidae (Subbotin *et al.*, 2005) suggests new opportunities for developing durable resistant rootstocks. The transformation of potato and banana germplasm to express a maize cystatin against nematode digestive proteinases and a peptide that interferes with chemoreception produced plants resistant to cyst and burrowing nematodes, respectively (Lilley *et al.*, 2011; Roderick *et al.*, 2012).

Diagnosis and sampling

Laboratories traditionally obtained samples to depths of 120 cm to obtain roots most likely to contain high densities of the nematode (Poucher *et al.*, 1967). It has since been demonstrated that processing a larger amount of roots from near the soil surface (that are acquired easily and inexpensively with a shovel) can detect nematode-infected trees more accurately than processing

the smaller amount of roots obtained deeper in the soil (Duncan *et al.*, 1994c). Visual stratification of orchards based on tree decline symptoms is important. Intensive sampling of suspicious trees increases the chance of detecting the nematode, whose densities can be quite low during some periods.

Pratylenchus

Three species of lesion nematodes, *P. coffeae*, *Pratylenchus brachyurus* and *Pratylenchus vulnus*, can damage citrus. *P. coffeae* is easily the most pathogenic (Fig. 12.5). It is widespread, having been reported on citrus in the USA (O'Bannon *et al.*, 1972), India (Siddiqi, 1964), Japan (Yokoo and Ikegami, 1966), Oman (Mani *et al.*, 1997), South Africa (Milne, 1982) and Taiwan (Huang and Chang, 1976). Variation among *P. coffeae* populations revealed it to be a species complex (Golden *et al.*, 1992; Duncan *et al.*, 1998, 1999). A lesion nematode, thought to be *P. coffeae*, detected on citrus in Sao Paulo State, Brazil, was



Fig. 12.5. Valencia orange trees on rough lemon rootstock in various stages of decline (note large numbers of replanted trees) due to infection by the lesion nematode *Pratylenchus coffeae*. (Photograph courtesy of L. Duncan.)

renamed *Pratylenchus jaehni* (Inserra *et al.*, 2001). It appears to be very similar to lesion nematodes from coffee in Sao Paulo (Duncan *et al.*, 1999), although the host ranges differ (Silva and Inomoto, 2002). *P. jaehni* is associated with unthrifty citrus trees. Cleopatra mandarin and Carrizo citrange, among other trifoliate hybrids, were found to be resistant to *P. jaehni* (Calzavara *et al.*, 2007; Bonfim *et al.*, 2011) but not to *P. coffeae* (O'Bannon *et al.*, 1976). Putative *P. coffeae* associated with native vegetation in Florida, which threatened the nematode-free certification of some citrus nurseries, were found to be genetically distinct from *P. coffeae*, incapable of reproducing on citrus, and were re-described (Inserra *et al.*, 1996, 1998; Duncan *et al.*, 1999).

Damage by *P. coffeae* has been observed in Florida and other regions (O'Bannon and Tarjan, 1985). In South Africa, the nematode has not been associated with economic problems (Milne, 1982). Infection occurs primarily in the feeder roots, where all motile stages of the nematode penetrate cortical tissue, but vascular tissues remain intact until invaded by secondary organisms. *P. coffeae* appears to be obligatorily amphimictic, with males feeding in the roots (Radewald *et al.*, 1971b; Inserra *et al.*, 2001). Reproduction is highest when soil temperatures are relatively high (26–30°C). At these temperatures, the life cycle is completed in less than 1 month and may reach density levels as high as 10,000 nematodes/g root (O'Bannon and Tomerlin, 1969b; Radewald *et al.*, 1971a), and can survive in roots in soil for at least 4 months.

In pot studies, *P. coffeae* reduced citrus root weights by as much as half (O'Bannon and Tomerlin, 1969b; Radewald *et al.*, 1971a). Growth reduction of young trees in the field ranged from 49 to 80%, and the numbers of fruit during the first bearing years ranged from threefold to 20-fold differences between infected and non-infected trees (O'Bannon and Tomerlin, 1973). Soil types ranging from sands to sandy loams did not affect the pathogenicity of *P. coffeae* (O'Bannon *et al.*, 1976). Reported migration of the nematode through soil was of the order of 1 m/year (Tarjan, 1971; O'Bannon and Tomerlin, 1973; O'Bannon, 1980), although the rate of spread of decline symptoms in groves was greater. The limited distribution of *P. coffeae* in Florida citrus is due partly to a rootstock certification programme,

and may also be due to competition with the more widespread *T. semipenetrans* (Kaplan and Timmer, 1982). No commercial rootstocks resistant to the nematode are available.

P. brachyurus has a biology similar to that of *P. coffeae*. In Florida, the nematode was present in 90% of the groves sampled (Tarjan and O'Bannon, 1969), while it has not been reported from citrus groves in South Africa, even though it is widespread in that country (Milne, 1982). It is a proven pathogen of seedlings in greenhouse trials (Brooks and Perry, 1967; Tarjan and O'Bannon, 1969; Radewald *et al.*, 1971a; Tomerlin and O'Bannon, 1974) and on young trees in the field (O'Bannon *et al.*, 1974). It is generally not considered to be a problem on mature citrus (O'Bannon *et al.*, 1974). When *P. brachyurus* in mature Valencia orange trees on rough lemon rootstock was controlled with aldicarb, trees suffered less frost damage during a severe winter and subsequent yields were increased (Wheaton *et al.*, 1985).

P. vulnus has been found associated with citrus in Italy (Inserra and Vovlas, 1974) and California (Siddiqui *et al.*, 1973), and was shown to be capable of causing severe damage to nursery seedlings (Inserra and Vovlas, 1977). Biology, population growth rates and root damage are similar to those described for *P. coffeae*.

Belonolaimus longicaudatus

Belonolaimus longicaudatus can damage citrus by greatly reducing the fibrous root abundance of trees. Sting nematodes occurred in fewer than 10% of Florida citrus orchards (Esser *et al.*, 1993), but their incidence in regions with sandy soil was estimated to be as high as 64% (Duncan *et al.*, 1996). Sting nematodes are widely distributed on a number of cultivated and non-cultivated host plants in south-eastern USA, and the group comprises a species complex (Han *et al.*, 2006; Gozel *et al.*, 2006a). The nematode is ectoparasitic, feeding on the root tips of citrus. The root systems of infected trees appear very coarse, due to a reduction in the number of lateral roots and swollen fibrous roots (Fig. 12.6). Fibrous roots also have swellings at or near terminals, as well as multiple apices. Sting nematodes are associated with severe stunting of trees on all known rootstocks in the field (Standifer and Perry, 1960; Esser and Simpson, 1984;

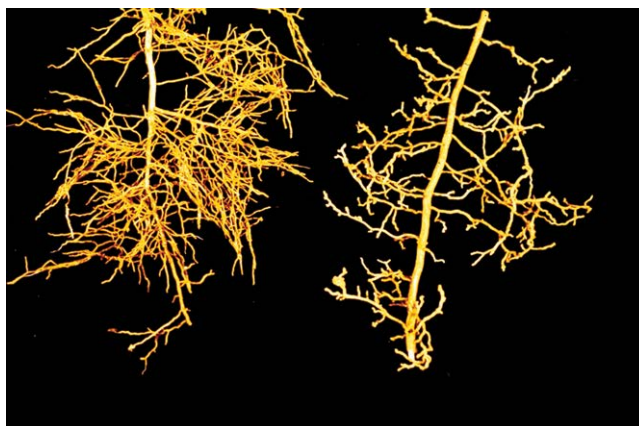


Fig. 12.6. Stubby root tips and reduced fibrous root system due to feeding by the sting nematode *Belonolaimus longicaudatus*. (Photograph courtesy of L. Duncan.)

Duncan *et al.*, 1996), and cause similar symptoms in pot experiments (Standifer and Perry, 1960; Abu-Gharbieh and Perry, 1970). The economic importance of sting nematodes may be increasing due to changing cultural practices that favour maintaining a cover crop in the row middle. Newly planted orchards often contain patches of stunted trees (Fig. 12.7). Tree condition and yield in these orchards are related inversely to the population density of sting nematodes. Growth of the stunted trees usually remains poor for several years, after which they resume normal growth. Soil water potential beneath heavily infected young trees with few roots is consistently higher than beneath lightly infected trees with dense roots and high transpiration rates. Thus, when young trees are planted in locations with high densities of sting nematodes, roots are damaged continually until the trees manage to develop a root system dense enough to cause nematodes to move deeper into the soil due to periodic moisture deficit in surface soil (Duncan *et al.*, 1996).

Pre-plant soil fumigation and post-plant nematicide treatments have alleviated symptoms of sting nematode parasitism (Bistline *et al.*, 1967).

Meloidogyne

Root knot nematodes (*Meloidogyne* spp.) capable of attacking citrus are very limited in distribution.

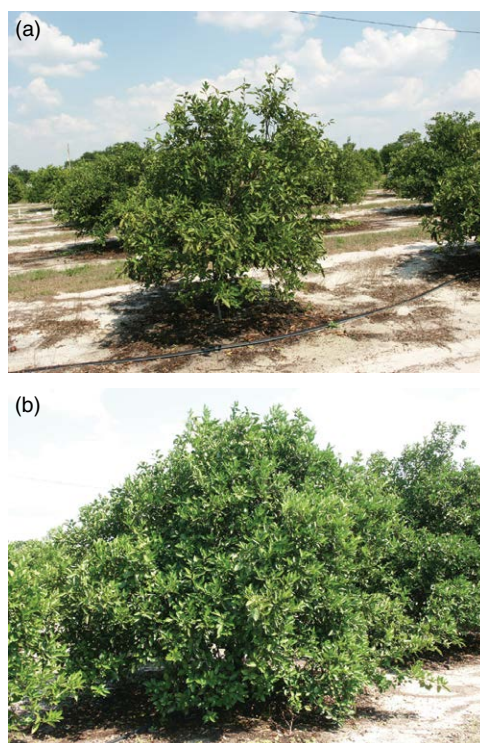


Fig. 12.7. The effect of sting nematodes on 8-year-old citrus trees on Swingle citrumelo rootstock; (a) planted in an area of the orchard infested heavily with sting nematodes (1.6 m height); (b) planted in a lightly infested area of the same orchard (2.2 m height). (Photograph courtesy of L. Duncan.)

Although there have been several reports of the common species, *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne arenaria*, developing or reproducing on citrus (Minz, 1956; Den Ouden, 1965; Whitehead, 1968; Scotto la Massèse, 1969; de Brito *et al.*, 2000), they appear to be a problem in only a few localized regions in China and the Far East. An apparently pathogenic species was reported from Taiwan and New Delhi, where it caused elongated galls on citrus roots. The nematode was given the common name 'Asiatic pyroid citrus nematode' and was found to be able to complete its life cycle on several citrus and other plant species, including maize and sweet potato. *Meloidogyne fujianensis* (Pan, 1985) and *Meloidogyne oteifae* (Pan, 1984) have been reported from China on *C. reticulata*, with the former species parasitizing up to 60% of the citrus trees surveyed.

A more common situation in which root knot nematodes may cause problems in citrus was reported by Van Gundy *et al.* (1959), who found that *M. incognita*, *M. javanica* and *M. arenaria* infected roots of Troyer citrange and sour orange, causing small galls, but without reproducing. Galls on plants in the field were associated with unthrifty plant growth, but were found to be due to infection by populations that were supported on weed hosts. This work was later supported by Inserra *et al.* (1978) and Orion and Cohn (1975), who observed extensive root damage due to invasion of citrus roots by *M. javanica*, even though no reproduction occurred.

Xiphinema

A large number of nematode species of the genus *Xiphinema* have been reported from the citrus rhizosphere (Gozel *et al.*, 2006b). Very little research has been undertaken regarding the pathogenicity of these nematodes to citrus, however, even though high densities of some species have been consistently associated with citrus in California, South Africa and Sudan (Yassin, 1974; Cohn, 1976; Milne, 1982). Most species of *Xiphinema* predominate in lighter-textured soils (Cohn, 1969). In Sudan, high densities of *Xiphinema brevicollum* were associated with declining grapefruit trees. Subsequent pot studies resulted in similar root symptoms of

stubby, swollen roots, and root abundance was reduced greatly by the nematode (Yassin, 1974). Similarly, high densities of *Xiphinema vulgare* are associated with declining citrus trees in Florida, and caused necrosis and severe reduction of the root systems of seedlings in pots (Leone *et al.*, 1997). The nematode was shown to reproduce on citrus, but required 274 days at 24°C to complete its life cycle (Coiro *et al.*, 2002). *X. vulgare* prevalence and apparent damage to trees in Florida has increased dramatically, but for unknown reasons (Gaver *et al.*, 2011). *X. brevicolle* and *Xiphinema index* reduced sour orange seedling size by nearly half in pot studies in Israel (Cohn, 1970; Cohn and Orion, 1970).

Trichodorus* and *Paratrichodorus

Low densities of *Trichodorus* and *Paratrichodorus* spp. are often encountered in soil samples from citrus (Baines *et al.*, 1959; Malo, 1961; Colbran, 1965). *Paratrichodorus lobatus* has also been found in high densities in citrus nurseries in Australia, where it is widespread in nurseries and orchards (Stirling, 1976). *Paratrichodorus porosus*, *P. lobatus* and *Paratrichodorus minor* have been reported to reduce root elongation and cause stubby root symptoms without evidence of necrosis on citrus in pot studies (Standifer and Perry, 1960; Stirling, 1976). Despite decreasing feeder root weight in a pot study, *P. lobatus* did not affect taproot or seedling weights, nor were population densities in a nursery correlated with tree size (Stirling, 1976).

Hemicyclophora* and *Caloosia

Hemicyclophora arenaria is a species native to plants in the desert valleys of southern California that causes damage in citrus nurseries (McElroy *et al.*, 1966). The nematode was closely studied (Van Gundy, 1959; Van Gundy and Rackham, 1961) and quarantined to prevent its spread to other areas of that state. The nematode feeds in large numbers at root tips whose roots typically develop around galls arising from hyperplasia. *Caloosia nudata* causes similar symptoms on citrus in Australia (Colbran, 1963).

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13 Nematode Parasites of Subtropical and Tropical Fruit Tree Crops*

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This chapter covers subtropical and tropical fruit and nut tree crops, as well as vines, for many of which detailed information concerning nematode damage is relatively scarce. We have included here 22 tree and vine crops that, by virtue of their production value on a world basis, their importance in world trade or their local significance at the present time, may largely be regarded in this context as major crops among the long list of tropical and subtropical fruit cultivated worldwide. These include eight fruit tree crops, three nut crops and eleven vine crops. In this edition, we have included grapes into the vine crops section, due to the cultivation increase of grapevines for wine, raisins and table grape production in subtropical areas. In all crops, we have emphasized those nematode pests for which some evidence of economic impact exists. The crops are reviewed herein in alphabetical order of their common name within each section.

Fruit Crops

Avocado

Plant parasitic nematodes that have been associated with avocado (*Persea americana*) are

Rotylenchulus reniformis, *Criconebella* sp., *Meloidogyne hapla*, *Pratylenchus brachyurus*, *Pratylenchus coffeae* and *Radopholus similis*. In particular, *P. brachyurus* and *R. similis* (citrus race) can reduce the performance of avocado trees, and can create root lesions. In a greenhouse trial where avocado trees were challenged with different levels of *R. similis*, this nematode caused light brown to black root lesions, as well as root rotting over time (Jasy and Koshy, 1992). *Xiphinema brevicolum* has been shown to reduce seedling growth in pots (Cohn, 1968). Sher (1955) attributed plant damage in California to *Pratylenchus vulnus*, and reduced tree growth was shown to be caused by this nematode, both in greenhouse inoculation experiments and in pre-plant fumigation trials (Sher *et al.*, 1959).

To date, little is known about nematode economic thresholds in avocado and the correlated impact on yield and overall productivity of the orchard. However, a report from South Africa indicated that the use of a granulate, non-fumigant nematicide reversed avocado decline compared to an untreated treatment (Willers, 1999). Another report from South Africa showed that mulching with composted chips, eucalyptus chips or grass in 3-year-old avocado trees reduced the size of plant parasitic nematode populations (Nzanza and Pieterse, 2013). Avocado

*A revision of the chapter written by F.E. El-Borai and L. Duncan in the second edition.

rootstock diversity that could be nematode tolerant or resistant is yet to be described. So far, Milne and Keetch (1976) reported avocado to be a non-host for the banana race of *R. similis* in Natal, South Africa. Interestingly, most of the economically important sedentary plant nematodes do not appear to be a serious problem on avocado. There is no evidence that *R. reniformis* or *M. hapla* cause economic damage to avocado. The genera *Helicotylenchus* and *Meloidogyne* were encountered with some frequency at population densities between 150 and 350 nematodes/100 ml of soil in a survey of avocado nurseries and orchards in Colombia (Saltaren *et al.*, 1999). Although *M. incognita* race 2 failed to reproduce or cause galling on potted seedlings, inoculation with *Helicotylenchus dihystra* reduced seedling growth by 20–50% (Saltaren *et al.*, 1999), suggesting a need for further study of the economic importance of ectoparasitic nematodes in avocado.

Fig

Root knot nematodes are the most severe nematode problem in fig (*Ficus carica*) cultivation, and certainly the best documented. Among the identified species are *Meloidogyne arenaria*, *Meloidogyne incognita*, *Meloidogyne acrita* and *Meloidogyne javanica* (McSorley, 1981). In Brazil, Medina *et al.* (2006) characterized *M. incognita* races 1, 2 and 3 in fig orchards, identifying race 1 as the most abundant. Root knot nematodes can cause fig root swelling (Kepenekci *et al.*, 2006) and can be found in orchards with fig decline (Hosomi and Uchiyama, 1998).

Heterodera fici is another important nematode of fig, and is widely distributed throughout the world. Leachates from fig roots stimulate egg hatch and the emergence of juveniles from cysts (Di Vito and Sasanelli, 1990). The potential pathogenicity of *H. fici* on fig seedlings was demonstrated in pot trials by Di Vito and Inserra (1982), who reported 30% death of plants with an initial nematode population of 8 eggs and juveniles/ml of soil, and 100% plant mortality with an initial nematode density of 64 eggs and juveniles/ml of soil and higher. Thus, while field populations of *H. fici* do not generally appear to attain such damaging levels in orchards, the nematode can be considered a potential threat in

fig nurseries, where fig rootstocks are often obtained from seeds. It is also noteworthy that the nematode has caused considerable damage to potted plants of the related *Ficus elastica* (Scotto La Massè *et al.*, 1984; Narbaev and Sidikov, 1985).

Fig is also a host of *Xiphinema index*. The anatomical changes caused by this nematode on fig roots – in the form of terminal galls and modified cells – as well as the associated biochemical changes have been studied and described in great detail (Poehling *et al.*, 1980; Wyss *et al.*, 1980), as has the feeding behaviour of the nematode on fig roots (Wyss, 1987). Although fig has been shown to be a more favourable host of *X. index* than grapevine (Coiro and Lamberti, 1978; Malan and Meyer, 1999), there does not appear to be as much damage to plant growth. Nevertheless, the nematode is considered of economic importance in California, where trees respond favourably to pre-plant fumigation (Koenning *et al.*, 1999). There is no known virus transmission in fig by this nematode, which is the vector of fanleaf virus disease in grapevine. Other nematode species possibly associated with injury to fig roots are *Paratylenchus hamatus*, *P. vulnus*, *P. coffeae* and *Helicotylenchus* spp.

Several measures have been recommended to reduce nematode damage to fig production. Pre-plant fumigation permits better establishment of newly planted trees (Krezdorn and Adriance, 1961). Partial nematode control and improved rooting on cuttings under nursery conditions can be attained by the application of systemic nematicides. Nematicide application to control *M. incognita* and *H. fici* increased the number of fruit per plant of fig cv. Roxo of Valinhos (Pereira *et al.*, 2010). Work has also been carried out to find root knot resistant fig rootstocks. Tests in California revealed that while all *F. carica* specimens examined were susceptible to *Meloidogyne* spp., four other *Ficus* species (*Ficus racemosa*, *Ficus coccinifolia*, *Ficus gnaphalocarpa* and *Ficus palmata*) showed a high degree of resistance to unidentified species of root knot nematodes, as well as good graft compatibility with *F. carica* (Krezdorn and Glasgow, 1970). The commercial cultivar 'LSU Purple' is reported to be nematode resistant (O'Rourke, 1992). Twenty rootstocks grown in 'sick' soils exhibited a range of root galling, but growth of the scion 'Masui Dauphine' (used on all rootstocks) was

not correlated with gall index. The rootstock 'Zidi' performed well in a number of field trials (Hosomi *et al.*, 2002). In Brazil, different fig genotypes tested were susceptible to *M. javanica*, *M. incognita* and *Meloidogyne enterolobii* (Costa *et al.*, 2015). The fig cultivars 'Celeste' and 'Poulette' are considered resistant to the nematode, while the species *Ficus glomerata* can exhibit a high degree of tolerance, but may have other unsatisfactory qualities as a rootstock.

Guava

The best documented nematode problem affecting guava (*Psidium guajava*) is that created by *Meloidogyne* spp., which is a recognized limiting factor in commercial guava production in tropical America. Important species are *M. incognita*, *M. arenaria*, *M. hapla* and *M. javanica* (Fig. 13.1). Willers (1997) and Carneiro *et al.* (2001) identified *Meloidogyne mayaguensis* (also known as *M. enterolobii*) as the cause of severe guava decline. New plantings can become non-productive within 5 years (Shesteporov, 1979). In addition, *H. dihyssera*, *P. brachyurus*, *R. reniformis*, *Mesocriconema* sp., *Hemicriconemoides strictathecatus*, *Paratylenchus* sp., *Tylenchorhynchus contractus*, *Xiphinema brevicolle* and *Monotrichodorus monohystera* have also been associated with guava orchards (Castellano *et al.*, 2012).

Screening of several *Psidium* species for possible resistant rootstocks has been performed. The rootstock *Psidium friedrichstalianum* showed a high degree of resistance to *Meloidogyne* spp. (Fernandez Diaz-Silveira, 1975). Mathus *et al.*

(1999) found that *M. incognita* populations were not supported by *P. friedrichstalianum*, and the tolerance limit of the rootstock was 60-fold lower than that of a *P. guajava* cultivar. However, the reaction appears to vary with plant material or nematode population. Gonzalez and Sourd (1982) and Villota and De Agudelo (1997) found *P. friedrichstalianum* to show only moderate tolerance to *Meloidogyne*. Other *Psidium* species – among them *Psidium cattleianum*, *Psidium molle* and *Psidium guineense* – and cultivars of *P. guajava* can be highly susceptible to the nematode.

Gomes *et al.* (2011) showed that progressive root rot in guava trees parasitized by *M. enterolobii* was caused by *Fusarium solani*. In the disease complex, known as guava decline, parasitism by the nematode predisposes *F. solani*-immune trees to extensive fungal root decay. *M. enterolobii*-parasitized trees may have galled roots and some root rot for several months without secondary symptoms on the shoots. Several strategies have been used to manage the nematode and one of the most effective was the use of soil organic amendments (Almeida *et al.*, 2012). Different combinations of soil and foliar fertilization (cow manure, neem oil cake, cultivation of mint and velvet bean) under the canopy of guava seedling reduced the population of the second-stage juveniles of *M. mayaguensis* (Souza *et al.*, 2006). The synergistic interaction between *F. solani* and *M. enterolobii* has been reported affecting guava in both Venezuela and Mexico (Suarez *et al.*, 1999).

Lychee

Detailed information on economic nematode damage to lychee (*Litchi chinensis*) is available only from South Africa. Milne (1982a) recognized *X. brevicolle* and *Hemicriconemoides mangiferae* as major nematode pests of lychee, causing a severe tree decline syndrome. Typical above-ground symptoms were the presence of many bare twigs and branches, leaf chlorosis, leaf-tip burn, poor flowering with excessive fruit drop and, in some orchards, up to 40% death of the trees. Root symptoms were severe stubby root and darkening of the roots, leading eventually to the loss of a large proportion of the feeder root mass and consequent interference in the uptake



Fig. 13.1. Guava roots infested with root knot nematodes from Niger. (Photograph courtesy of N. Greco.)

of nutrients and water. *X. brevicollum* feeds more superficially, while *H. mangiferae*, which causes extensive destruction of the cortical tissue, is considered the more severe pest. Populations of 40 *H. mangiferae*/ml of soil and roots and 20 *X. brevicollum*/ml of soil and roots were recorded. *M. javanica* can also infect lychee roots, although galls are generally inconspicuous. *Trichodorus* spp. have also been found associated with lychee nursery seedlings. Pre-plant soil fumigation with 1,3-dichloropropene can effectively improve the performance of lychee replants in nematode-infested areas.

Mango

The most widely distributed nematode associated with mango (*Mangifera indica*) is *H. mangiferae* (Siddiqi, 1977; McSorley, 1992), which has been shown to be damaging to mango seedlings at a population level of 6 nematodes/ml of soil (Saeed, 1974). The nematode is widespread in mango orchards and on numerous other crops in India, particularly in sandy soils, where population density is related strongly and positively to soil moisture (Ashokkumar *et al.*, 1991). Although the pathogenicity to mango of *H. mangiferae* has been demonstrated (McSorley, 1992), its economic importance in the field is unclear. McSorley *et al.* (1981) have reported a wide distribution and strong association between *H. mangiferae* and declining mango in Florida, but the relationship is not always evident (Ashokkumar *et al.*, 1991).

H. mangiferae was observed feeding on mango roots together with *X. brevicolle* in South Africa, but chemical treatment of the trees, while reducing nematode populations, failed to induce a favourable tree response (Milne, 1982b). Mango infested by *H. mangiferae* and *R. reniformis* responded strongly to treatment with systemic nematicides. Fenamiphos applications were found effective in controlling *P. brachyurus*, but not *R. reniformis*, in Florida (McSorley and Parrado, 1983). *R. reniformis* appears to be the only sedentary nematode that commonly infects mango (McSorley and Parrado, 1983) and, interestingly, soil and root populations on seedlings are reduced effectively by the application of the growth regulant ethephon (Badra and Khattab, 1982). Anwar *et al.* (1991) considered *R. reniformis* to be

damaging on mango, but McSorley *et al.* (1981) found no relationship between infestation by the nematode and tree decline symptoms. Although detected in soil associated with mango (Maqbool, 1991; McSorley, 1992), there is only one report documenting the infection of mango by *M. incognita* (Mani and Al Hinai, 1995). In 2015, *P. brachyurus* was reported for the first time in Brazil being associated with the roots of mango seedlings cv. Tommy Atkins in naturally infested soil in the Assu municipality (Torres *et al.*, 2015). The mango decline syndrome has been reported in southern Punjab mango plantations, and the plant parasitic nematodes and fungal pathogens associated with mango were reported to be the causal agent. Populations of *H. mangiferae*, *Macroposthonia* spp. and *R. reniformis* were very high. The authors speculated that the mango decline must have included *H. mangiferae* and *R. reniformis*, which predisposed the mango seedlings to infection by associated fungi that by themselves were weak pathogens (Anwar *et al.*, 2012).

Olive

Olive (*Olea europaea*) is a host to more than 100 species of plant parasitic nematodes in over 30 genera (Castillo *et al.*, 2010). Some nematode species, such as *Rotylenchulus macrosoma*, *Meloidogyne lusitanica*, *Meloidogyne baetica*, *Helicotylenchus oleae* and *Helicotylenchus neopaxilli*, are rarely encountered in other crops, whereas other nematodes such as *Pratylenchus* spp. and *M. javanica* (Fig. 13.2) are commonly found elsewhere (Belahmar *et al.*, 2015; Ali *et al.*, 2016). Nevertheless, olive is an extremely vigorous plant that thrives in hilly, relatively dry areas, where most groves are situated. Under such conditions, nematodes generally occur in small numbers and are apparently of limited economic importance. Also, in old orchards, trees are grafted on wild olive rootstocks that may tolerate nematodes to some extent. In newer orchards, plants are derived from cuttings of various cultivars that are more tolerant of nematode damage. For example, the olive cv. Zard is less susceptible to *M. incognita* than to *M. javanica* (Afshar *et al.*, 2014). In olive production regions, root knot nematodes can occur patchily or in sporadic distributions in both nurseries and established



Fig. 13.2. *Meloidogyne javanica* root galling on olive cv. Arbequina in Spain. (Photograph courtesy of P. Castillo.)

orchards (Castillo *et al.*, 2010), where they can cause heavy root galling (Fig. 13.2). Root knot nematodes have been shown to reduce seedling growth drastically in inoculation trials (Lamberti and Baines, 1969a). Indeed, tolerance limits of 0.49–0.61 eggs/ml soil and minimum yields near 50% were estimated for the effects of *M. javanica* on seedling growth of two olive cultivars (Sasanelli *et al.*, 2002). *M. arenaria* race 2, *M. javanica* and *M. incognita* race 1 caused growth reduction, yellowing and leaf drop in seedlings of two common olive rootstocks (Nico *et al.*, 2003). *M. baetica* is a species from wild olive in Spain that does not develop on several well-known root knot hosts, such as tomato, chickpea and pea (Castillo *et al.*, 2003b). The economic importance of *M. baetica* is unknown, but it infects common olive rootstocks, and its histopathology and population development on olive are similar to those reported for other root knot species. Some olive cultivars reported to be resistant to *M. javanica* are Coratina, Manzanillo and Leccino (Abbas and Mohammad, 2005). Lamberti *et al.* (2001) found some evidence that infection by

M. incognita enhanced damage by the wilt-inducing fungus *Verticillium dahliae*, whereas Palomares-Rius *et al.* (2016) indicated that root knot infection did not breakdown resistance to this soil-borne pathogen in selected clones of wild olive.

P. vulnus has been implicated as a factor in olive decline and has been demonstrated in inoculation trials as a potential parasite of olive (Lamberti and Baines, 1969b). Nico *et al.* (2002, 2003) found that *P. vulnus* and *Pratylenchus penetrans* reduced seedling growth in pot experiments. Several species of *Helicotylenchus*, particularly *H. dihystra*, *Helicotylenchus digonicus*, *Helicotylenchus erythrinae* and *H. oleae* have been observed to cause root necrosis and are considered to be capable of affecting olive tree growth. Species of *Xiphinema* also commonly occur around olive roots, and *Xiphinema elongatum* and *X. index* have been shown to affect olive plant growth (Sasanelli *et al.*, 1999). Recently, *Xiphinema* spp. was described as the only ectoparasite to have the potential to damage olive in Algeria (Belahmar *et al.*, 2015).

A number of rather specialized sedentary plant nematodes attack olive. A biotype of the citrus nematode *Tylenchulus semipenetrans* infects olive in California, Italy and Chile, and although population levels on olive are usually lower than on citrus (Inserra and Vovlas, 1977; Sepúlveda-Chavera *et al.*, 2015), unusually high levels of *T. semipenetrans* have been shown to inhibit olive growth (Lamberti *et al.*, 1976; Westerdahl and McKenry, 2016). *Trophotylenchulus saltensis* was described from olive roots in Jordan (Hashim, 1983b), and a very specialized cyst nematode, *Heterodera mediterranea*, first recorded from Italy, was shown to be capable of feeding and multiplying on olive roots, in which it formed syncytia and caused disorder of the stellar structure (Vovlas and Inserra, 1983). The first naturally occurring infestation of olive orchards by *H. mediterranea* was detected in Spain (Castillo *et al.*, 1999), and although visual symptoms were unapparent, the nematode was capable of reducing growth of the cultivar 'Arbequina', but not 'Picual', in pot studies (Castillo and Vovlas, 2002). Two sedentary ectoparasitic nematode species, *Gracilacus peratica* and *Ogma rhombosquamatum*, have been observed to feed on olive roots, and their feeding behaviour has been described in detail (Inserra and Vovlas, 1977; Vovlas and Inserra, 1981); however, there is no evidence of a pathogenic effect. Similarly, three species of *Rotylenchulus* have been studied in detail on olive, namely *Rotylenchulus macrodoratus* (Inserra and Vovlas, 1980), *R. macrosoma* (Cohn and Mor, 1988) and *R. reniformis* (Hirschmann *et al.*, 1966). Wild olive orchards heavily infested by *R. macrosoma* were discovered for the first time in Spain (Castillo *et al.*, 2003a). A threshold of 0.5–1.0 *R. macrosoma*/ml has been reported for olive (Castillo *et al.*, 2010).

Measures for practical nematode control in olive have been limited so far to nurseries, where pre-plant fumigation has been recommended for control (Hashim, 1982; Westerdahl and McKenry, 2016). Suggestions for bare root dips of seedlings in suspensions of nematicidal chemicals (such as fenamiphos), prior to transfer into groves, have also been offered for reducing root knot nematode infestation (Lamberti and Di Vito, 1972). In California, various soil solarization techniques were as effective as soil fumigation to disinfest olive nursery soils of *T. semipenetrans*, *P. vulnus* and *Mesocriconema xenoplax* (Stapleton

et al., 1999). Non-fumigant nematicides have been shown to be effective in controlling root knot nematodes in 1-year-old olive seedlings (Soltani *et al.*, 2013). Resistance or tolerance of some olive cultivars to *P. vulnus*, *R. reniformis* and various species of *Meloidogyne* has been reported (Al-Sayeed and Abdel-Hameed, 1991; Pinochet *et al.*, 1992; Robinson *et al.*, 1997), and improved methods to screen olive for nematode resistance have been developed (Sasanelli *et al.*, 2000). Biological control of nematodes in olive has been evaluated. Under field conditions, the nematode-trapping fungus *Dactylaria brochopaga* applied twice as a powder or liquid (2 kg or 2 litres/tree) reduced *M. incognita* on the olive cv. Manzanella (Aboul-Eid *et al.*, 2006). Under controlled conditions, arbuscular mycorrhizal fungi colonization significantly reduced the severity of root galling by 6.3–36.8%, as well as reproduction by 11.8–35.7% of both *M. incognita* and *M. javanica* (Castillo *et al.*, 2006). A biological product containing *Myrothecium verrucaria* controlled *T. semipenetrans* in olive in Chile (Sepúlveda-Chavera *et al.*, 2015).

Papaya

Of the several nematodes reported to be associated with papaya (*Carica papaya*), only two genera appear to be economically significant: the root knot nematode (*Meloidogyne* spp.) and the reniform nematode (*Rotylenchulus* spp.). Both nematode genera have a wide distribution in papaya-producing regions. In Hawaii, yield losses to these two genera are estimated to be 15–20% (Koenning *et al.*, 1999). It is also noteworthy that *H. dihystra* has been detected at densities as high as 200 nematodes/g of root in South Africa, although there are no subsequent reports of pathogenicity (Willers and Neething, 1994). Other nematodes, such as *P. brachyurus*, *Mesocriconema* sp., and *H. strictathecatus*, have also been associated with papaya orchards, although their economic importance is still not known (Castellano *et al.*, 2012).

Heavy root knot infections of papaya (Fig. 13.3.), primarily by *M. incognita* and *M. javanica*, have been reported from many countries (McSorley, 1981). Root galling is often severe, with galls formed the size of golf balls (Milne, 1982a). A tolerance limit of 0.16 eggs and

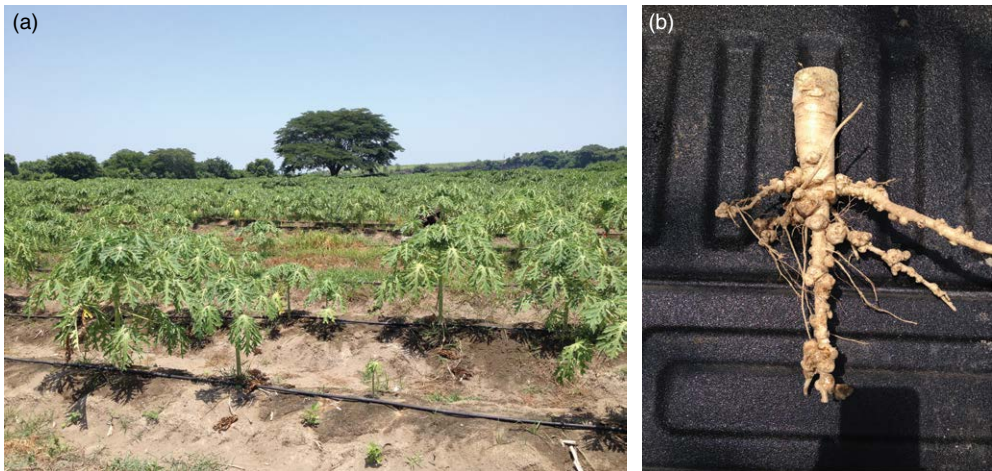


Fig. 13.3. Poor plant growth in a papaya field (a) and root galling (b) caused by *Meloidogyne incognita* in Mexico. (Photographs courtesy of A. Toledo.)

juveniles/ml of soil was estimated for *M. incognita* race 1 on papaya seedlings (Bustillo *et al.*, 2000). Other studies have reported similar levels of seedling damage by *M. incognita* (Ramakrishnan and Rajendran, 1998a). The root knot nematode causes severe damage in the field, producing root rot, reducing the life expectancy of the plant and drastically decreasing yield levels (Milne, 1982a).

Ramakrishnan and Rajendran (1998b) estimated nearly 40% fruit loss in the first crop after planting, with increasing losses likely thereafter due to *M. incognita*. Recommended control measures call for pre-plant soil fumigation, especially in seedbeds, and the selection of non-infested planting sites. Post-plant application of systemic nematicides such as carbofuran, aldicarb and fenamiphos effectively reduced root gall formation and increased plant growth and yield (Gupta and Yadav, 1988; Routaray and Das, 1988; Ramakrishnan and Rajendran, 1999). Although less successful than nematicides, organic amendments (particularly neem) and mulches provided some control of root knot nematodes and increased fruit yield (Routaray and Das, 1988; Khan *et al.*, 1997; Ramakrishnan and Rajendran, 1999; Elder *et al.*, 2002; Srivastava, 2002). Most cultivars of *C. papaya* are highly susceptible to *M. incognita* (Babatola, 1985; Iglesias and Perez, 1991). Closely related species such as *Vasconcellea quercifolia* and *Vasconcellea pubescens* (syn. *Carica candamarcensis*)

are also root knot susceptible (McSorley, 1981), although there is a discrepancy regarding *Carica cauliflora* (Indra and Rajvanshi, 2001; Rosales and Suarez, 2001). Two studies have found *C. papaya* cv. Pusa 22-3 to be resistant, and several other cultivars are reported to have varying degrees of resistance to *M. incognita* (Reddy *et al.*, 1988; Khan *et al.*, 1995; Ramakrishnan and Rajendran, 1998a,b). Severe *M. incognita* infestations of papaya and tomato were observed wherever they were intercropped in fields in north Yemen (R. Sikora, University of Bonn, Germany, 2016, personal communication).

Reniform nematode infection of papaya has also been commonly reported. *Rotylenchulus parvus* has been identified from Kenya, and an unidentified species of *Rotylenchulus* was reported to be associated with this crop in Thailand and Florida (McSorley, 1981). *R. reniformis* has been implicated in severe plant damage and yield reduction in Puerto Rico, and in Trinidad it has been associated with tree death and toppling. A survey of papaya in five Indian states detected 100% incidence of *R. reniformis* at population densities up to 1025 nematodes/100 g of soil (Ganguly *et al.*, 1997). In Fiji, severe damage by the nematode has been reported in nursery seedlings and young plants, and in Brunei plants have reportedly been killed by a combination of *R. reniformis* and *Phytophthora nicotianae* var. *parasitica*. In pot experiments, *R. reniformis* at 620 nematodes/200 ml of soil reduced the growth of

papaya seedlings (Karim, 1989). Ramakrishnan and Rajendran (1999) found that *M. incognita* was more virulent than *R. reniformis*, and competition between the two mitigated the virulence of *M. incognita*. Despite its widespread occurrence in papaya, there are few reports on management of the nematode in the field. Pre-plant soil fumigation in Hawaii with various nematicides effectively controlled the nematode and maintained low populations over periods of up to 6 months, with resultant yield increases in 15-month-old plants (Lange, 1960). Some cultivars of *C. papaya* appear to be resistant to *R. reniformis* (Patel *et al.*, 1989).

Persimmon

Little is known about economic nematode damage to persimmon (*Diospyros kaki*). Although the root knot nematode (*Meloidogyne* spp.) and the burrowing nematode *R. similis* have been reported to parasitize both *D. khaki* and *Diospyros virginiana* (McSorley, 1981; Sethi *et al.*, 1988; Inomoto *et al.*, 1991; Khurramov, 1993), no reports of actual plant damage by these nematodes appear to exist. *Pratylenchus scribneri* was reported to be the most frequently encountered nematode associated with persimmon in Korea (Park *et al.*, 1999).

The only nematode species associated with damage to the crop appears to be the citrus nematode *T. semipenetrans*, for which persimmon has been reported to be a very susceptible host. Extremely large soil and root populations of *T. semipenetrans* are commonly encountered in persimmon orchards in Israel on *D. virginiana* rootstock (Cohn and Minz, 1961), and have also

been observed in California on *D. lotus* rootstock (Nesbitt, 1956). A similar observation on *Diospyros lotus* roots was reported in Italy (Di Maio, 1979), where a resultant 20–30% loss in yield was estimated. Citrus nematode has also been reported from persimmon in Chile, New Zealand and Brazil (Gonzalez, 1988; Inomoto *et al.*, 1991; Knight, 2001).

Although no direct control measures appear to have been tested, it would seem probable that pre- and post-plant nematicide applications, as recommended in citrus cultivation, could effectively reduce *T. semipenetrans* populations on persimmon, if such treatments would be considered economically feasible. Other cultural control measures against the nematode in citrus groves could also be relevant to persimmon. No information is as yet available on the level of resistance to the nematode of the various persimmon rootstocks or other *Diospyros* species.

Nut Crops

Cashew

Plant parasitic nematodes that have been associated with cashew trees (*Anacardium occidentale*) are reported in Table 13.1. Although a few direct host–parasite relationships have been reported in this crop, no threshold levels are yet established. A review of cashew diseases in Brazil concluded that nematodes supported by the plant caused no evident damage (Freire *et al.*, 2002). *Xiphinema ifacolum* suppressed the growth of several tree crops in nurseries in Liberia, but not cashew (Lamberti *et al.*, 1992). It was

Table 13.1. Plant parasitic nematodes associated with cashew trees listed by country.

Plant parasitic nematodes	Country
<i>Criconeimoides</i> sp., <i>Scutellonema</i> sp., <i>Xiphinema</i> sp. and <i>Xiphinema index</i> ^a	Brazil
<i>R. reniformis</i>	Costa Rica
<i>Helicotylenchus astriatus</i> , <i>Helicotylenchus dihystra</i> , <i>Helicotylenchus elegans</i> , <i>Hemicriconeimoides mangiferae</i> , <i>Hemicyclophora attapadii</i> , <i>Hoplolaimus indicus</i> , <i>Meloidogyne incognita</i> , <i>Pratylenchus coffeae</i> , <i>Pratylenchus brachyurus</i> , <i>Rotylenchulus</i> <i>reniformis</i> , <i>Scutellonema brachyurus</i> , <i>Tylenchorhynchus leviterminalis</i> , <i>Xiphinema</i> <i>brevicollis</i> and <i>Xiphinema neoamericaum</i>	India
<i>Helicotylenchus coffeae</i> , <i>Meloidogyne</i> sp., <i>Radopholus</i> sp. and <i>Rotylenchulus reniformis</i>	Nigeria

^aNote: This nematode is reported to cause ‘xifinematose’, a common cashew disease in north-east Brazil, the economic impact of which is currently unknown.

believed that cashew was immune, or at least highly resistant, to different populations of the root knot nematodes in West Africa and in Brazil. However, a recent study in Nigeria showed that *M. incognita* reduced the height of cashew seedlings in the nursery (Orisajo, 2012). Besides the use of nematicides for managing nematodes in cashew production, leaf extracts made from *Hunteria umbellata* and *Mallotus oppositifolius* have been tested. These extracts caused *in vitro* mortality of *M. incognita* second-stage juveniles, and under greenhouse conditions increased the plant height, shoot weight and root weight of cashew seedlings while reducing nematode populations compared to the untreated control (Okeniyi *et al.*, 2013).

Macadamia

Information about the occurrence, economic threshold levels and management of plant parasitic nematodes on macadamia (*Macadamia integrifolia* and *Macadamia tetraphylla*) is limited. *M. incognita* race 2 reduced various yield components of *M. integrifolia* cv. Beaumont and *M. tetraphylla* cv. Nelmak under controlled conditions in South Africa (Mamphiswana *et al.*, 2011). In Brazil, the lesion nematode *P. brachyurus* was found in root samples collected in a macadamia field, although the trees were asymptomatic (Bonfim-Júnior *et al.*, 2012). It might be that macadamia trees have a certain level of tolerance or resistance to nematode damage, thus sustaining plant parasitic nematode communities in the roots while producing acceptable yield.

Pistachio

So far, there are over 29 different nematode species associated with pistachio (*Pistacia vera*) worldwide (Fatemy, 2009). In Iran, *Meloidogyne* is one of the most important nematode genera restricting pistachio production, and highly virulent pathotypes of *M. incognita* are suspected to be present (Madani *et al.*, 2012). Pistachio galled roots with *M. javanica* have been found in declining trees (Banihashemi and Kheiri, 1995), and an increasing gall and egg mass index has been

correlated to significantly reduced growth of the cultivars 'Ohadi' and 'Badami' (Salmani *et al.*, 2012). In California, McKenry and Kretsch (1984) surveyed pistachio orchards for plant parasitic nematodes and commonly found *P. hamatus*, *Pratylenchus neglectus* and *Xiphinema americanum*. *Meloidogyne* spp. were recovered in a minority of the orchards. They concluded that plant parasitic nematodes did not present a serious problem to pistachio production in California. However, current evaluation of rootstocks to lesion and root knot nematode populations in California suggest that, on sandy soils, these nematodes might affect pistachio yield negatively, although damage thresholds have not yet been determined. In Turkey, *Pratylenchus* spp. and *Helicotylenchus* spp. were the most abundant and frequent nematodes found in a 3-year survey (Yıldız and Elekçioğlu, 2011). *Pratylenchus* spp., *Xiphinema* spp. and *Criconea* spp. were also found at a lower frequency.

Pistachio growers often use species of *Pistacia* other than *P. vera* as rootstocks. Some of these species, particularly *Pistacia atlantica* and *Pistacia terebinthus* have some resistance to *M. javanica* (Anon., 1975) and possibly to other root knot species (McKenry and Kretsch, 1984), although root galling does occur. *P. vera*, *P. atlantica* and *P. terebinthus* are hosts of *P. vulnus*, but there is no evidence of economic importance (Pinochet *et al.*, 1992). This information indicates that adequate rootstock selection is a key component of plant parasitic nematode management in pistachio production. In addition, a combined resistance or at least a tolerant rootstock to Verticillium wilt, salinity and cold injury, such as UCB1, can be a helpful tool, especially if compared to an own-rooted *P. vera* cv. Kerman, which has nematode and *Phytophthora* spp. susceptibility (Fatemy, 2009). Carbamate non-fumigant nematicides are known to control plant parasitic nematodes in pistachio orchards (Maqbool and Qasim, 1988). More recently, biological control with *Pochonia chlamydosporia* var. *chlamydosporia* reduced by 56% the total population of *M. javanica* on pistachio cv. Kalehghochi (Ebadi *et al.*, 2009). Certain Iranian strains of *Pseudomonas* spp. caused 99% mortality in 72 hrs to *M. incognita* in *in vitro* tests, and reduced pistachio root galling and nematode multiplication in a greenhouse test (Khatamidoost *et al.*, 2015).

Vine Crops

Grape

Grapes (*Vitis vinifera*) are known to host a variety of plant parasitic nematodes. At least 162 different species have been reported from grapevines (Lamberti, 1988), although not all are economically important. Nicol *et al.* (1999) considered that the most important nematodes in grape production were *Meloidogyne* spp., *Xiphinema* spp., *T. semipenetrans*, *Pratylenchus* spp. and *M. xenoplax*, which have been found in several grape-growing regions worldwide. Root infection by plant parasitic nematodes can have a direct impact on water, nutrients and phytohormones levels that will further negatively influence chlorophyll, photosynthesis, respiration and the plant's energy (Melakeberhan and Webster, 1993). This imbalance affects growth, leaf area, yield and overall vineyard productivity.

Meloidogyne species are endoparasitic and can cause severe root galling (Fig. 13.4). They also can interfere with nutrient and water uptake (McKenry and Bettiga, 2013), and have been shown to cost about a half a calorie per individual to the vine (Melakeberhan and Ferris, 1989).

Xiphinema species are ectoparasitic nematodes that can reduce yield. Species such as *X. index* and *X. americanum* can also transmit Grapevine fanleaf virus and Grape yellow vein virus, respectively, which currently cannot be controlled at the field level (McKenry and Bettiga, 2013). *T. semipenetrans* causes no obvious symptoms of damage (Raski and Krusberg, 1984); however, it can reduce vigour and yield (McKenry and Bettiga, 2013). *Pratylenchus* spp. can interrupt the vine's nutrient flow, kill small roots and reduce growth (McKenry and Bettiga, 2013). *M. xenoplax* is an ectoparasitic nematode that feeds on roots, reducing water and nutrient uptake, increasing vine stress and thus causing yield reduction (Fig. 13.5) (McKenry and Bettiga, 2013). This nematode can reduce fine root



Fig. 13.4. *Meloidogyne incognita* root galling on own-rooted Chenin blanc grapevines (a) and a vineyard (b) showing poor growth patches (grey areas) due to the severe root knot nematode infestation, in Baja California, Mexico. (Photograph courtesy of J. Guevara.)

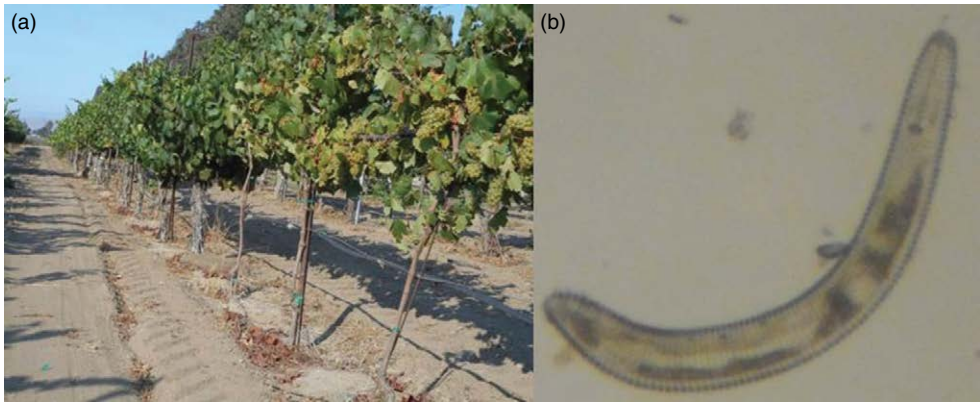


Fig. 13.5. Poor trunk and canopy growth (a) caused by *Mesocriconema xenoplax* over time in Chardonnay vines, and the ring nematode (b) found in the vineyard's soil in California, USA. (Photograph courtesy of J.A. Cabrera.)

production on grapes as well as diminish arbuscular mycorrhizal fungi colonization (Schreiner *et al.*, 2012), a fungus that can be considered beneficial to the grapes (Li *et al.*, 2002).

In California vineyards, *Meloidogyne* spp. (Fig. 13.6) soil populations are considered high at >500 nematodes/kg of soil in October–March and >200 nematodes/kg soil in March–October, whereas for *X. americanum* critical numbers are >200 and >100, respectively (McKenry and Bettiga, 2013). Independent of the time of the year, high populations for other nematodes were listed as: >100 for *P. vulnus*; >3000 for *T. semipenetrans*; >500 for *M. xenoplax*; and >200/nematodes/kg soil for *X. index*. In Australia, initial densities of *M. javanica* of 200, 400, 600 and 800 J2/kg of dry soil all reduced the yield of grape, and there was a strong correlation between increasing initial nematode densities and reduced yield (Rahman *et al.*, 2012). In an Australian Shiraz vineyard, the yield was 15% lower when *T. semipenetrans* was present at an average of 11,000 nematodes/kg compared to 932 nematodes/kg of dry soil (Rahman *et al.*, 2008). Damage threshold levels, however, also depend on cultivar and/or rootstock type (Quader *et al.*, 2002).

Yield losses due to plant parasitic nematodes have been estimated at 20% in California (Raski, 1986), and 7–20% in Australia, depending on the cultivar (Stirling *et al.*, 1992; Rahman *et al.*, 2012). In particular, the Grapevine fanleaf virus transmitted by *X. index* can cause 80% grape yield loss and fruit quality reduction (Martelli and Savino, 1991; Andret-Link *et al.*, 2004). The application of nematicides can increase

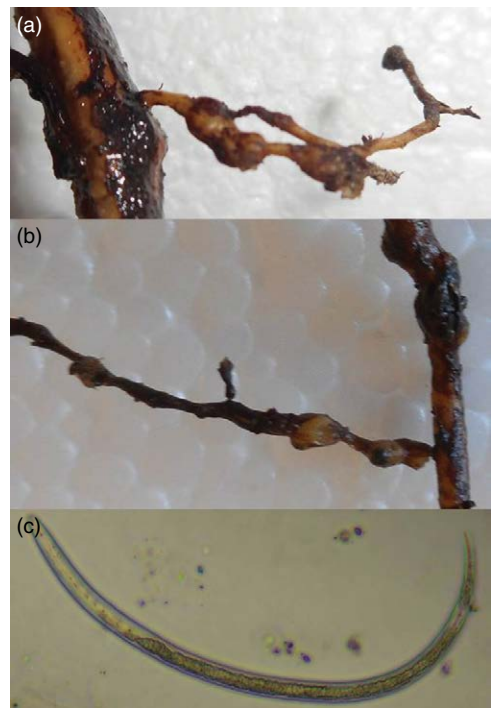


Fig. 13.6. Root galling caused by *Meloidogyne arenaria* on Flame grapes (a and b), and the second-stage juvenile recovered from the vineyard's soil (c), in California, USA. (Photograph courtesy of J.A. Cabrera.)

yield in vineyards where no obvious yield loss is observed. In vineyards infested with high numbers of *T. semipenetrans* (McKenry and Bettiga, 2013) or infested with two different species,

such as *Meloidogyne* sp. and *T. semipenetrans*, nematicide treatment caused a yield increase of 20–30% (Walker, 1989).

In some viticultural regions, soil fumigation is still permitted. Fumigants containing 1,3-dichloropropene alone, or mixed with chloropicrin, have been used effectively against nematodes before vineyard replanting (Cabrera *et al.*, 2011, 2012). Sodium tetrathiocarbonate and metham sodium have also been used. Modern pre-plant fumigation includes the use of plastics such as totally impermeable films (TIF) that can be used to reduce about 95% of chemical emissions and increase fumigant retention in the soil (Gao *et al.*, 2013, 2016). By using TIF or virtually impermeable films, the rates of 1,3-dichloropropene and chloropicrin could potentially be reduced from the full rate and still be effective towards nematodes and on certain soil-borne pathogens (Cabrera *et al.*, 2015). Reduced rates of 1,3-dichloropropene have also been shown to control *X. index* when applied as a post-plant treatment through drip lines in established vines, causing no phytotoxicity symptoms (Aballay and Merino, 2015). However, due to the cost of fumigation and environmental regulations, not all growers perform this practice.

A key tool for nematode management is the use of nematode-resistant or -tolerant rootstocks. For example, *Vitis berlandieri*, *Vitis riparia*, *Vitis rupestris* and many other *Vitis* species and their hybrids can be used. *Muscadinia rotundifolia*, a vine native to south-eastern USA, is another option that can be used for breeding rootstocks (Ferris *et al.*, 2012). In addition, there are certain *V. vinifera* cultivars, such as Black Champa, Dog Ridge and Degrasset that are resistant to nematodes such as *M. incognita* (Deshmukh *et al.*, 2004). The methods used to identify a nematode-resistant rootstock can vary (Cousins and Walker, 2001; Anwar *et al.*, 2002; Pinkerton *et al.*, 2005; McKenry and Anwar, 2006; McKenry and Bettiga, 2013). Overall, a resistant rootstock is a non- or poor host of the plant parasitic nematodes being tested. Interestingly, resistance to a nematode species does not intrinsically mean that it is resistant to the same species in other locations (Téliz *et al.*, 2007), or to other species. In addition, there have been reports of resistance breakdown in rootstocks in the field that were previously assigned as resistant under controlled screenings (Schreiner *et al.*, 2012). It is believed that there are specific nematode biotypes

in vineyard soils that can render a resistant rootstock ineffective (Cain *et al.*, 1984; Anwar *et al.*, 2000; Anwar and McKenry, 2002), perhaps due to a progressive nematode adaptation in the field to the rootstock resistance factors (Esmenjaud *et al.*, 2010). These nematode pathotypes are commonly referred to as 'virulent' or 'aggressive'. Another factor that could affect certain rootstock resistance is soil temperature (Ferris *et al.*, 2013). Other factors to consider before selecting a rootstock are their tolerance to salinity, drought, wet soil and lime, as well as phylloxera resistance. Moreover, vigour and yield needs to be considered if such information is available, since there are nematode-resistant rootstocks that provide better vigour and yield than others, even in the absence of nematode infestations (Moretti *et al.*, 2003; Pinkerton *et al.*, 2005). Tolerant rootstocks, those that can be infected by nematodes but still recover and yield well (Trudgill, 1991), can also be a valuable tool. Table 13.2 contains a piano chart of resistant and tolerant rootstocks to certain plant parasitic nematode populations that have been described previously. Grape breeding programmes for nematode resistance exist in different parts of the world. Modern molecular tools support the breeding process; for example, transgenic grapevine hairy root lines carrying an RNAi construct were shown to be less susceptible to nematode infection compared to normal roots (Yang *et al.*, 2013).

Another method used in the management of nematodes is fallow. Most literature dealing with fallow in vineyards has been directed toward the *Xiphinema* species, both because of their economic importance and their long-term survival in soil without feeding. The length of fallow is dependent on the initial density of nematodes in the soil (Helden *et al.*, 2014), as well as on the nematode species and soil type. A reduction in *X. index* densities required a fallow period of at least 4 years in the soils of Champagne and Provence, France (Demangeat *et al.*, 2005), over 5 years on a clay soil in Crete (Tzortzakakis, 2013) and 7 years in Bordeaux soils (Helden *et al.*, 2011). In California, a 10-year fallow is recommended to manage *X. index* and Grapevine fanleaf virus (McKenry and Bettiga, 2013). A study examining growing cover crops during fallow found that marigold and hairy vetch had the potential to reduce *X. index* under field conditions (Villate *et al.*, 2012). However,

Table 13.2. Piano chart of resistant or tolerant grape rootstocks to certain plant parasitic nematodes described in Nicol *et al.* (1999), Pinkerton *et al.* (2005), Ferris *et al.* (2012), Somavilla *et al.* (2012) and McKenry and Bettiga (2013).

<i>Xiphinema</i> <i>index</i>	<i>Meloidogyne</i> <i>incognita</i>	<i>Meloidogyne</i> <i>javanica</i>	<i>Meloidogyne</i> <i>arenaria</i>	<i>Pratylenchus</i> <i>vulnus</i>	<i>Meloidogyne</i> virulent pathotypes	<i>Tylenchulus</i> <i>semipenetrans</i>	<i>Mesocricone-</i> <i>ma xenoplax</i>	<i>Meloidogyne</i> <i>chitwoodi</i>	<i>Xiphinema</i> <i>americanum</i>	<i>Meloido-</i> <i>gyne</i> <i>hapla</i>
O39-16	99R	101-14Mgt	99R	101-14Mgt	101-14Mgt	110Richter	O39-16	RS-3	6-19B	Ramsey
110Richter	101-14Mgt	110Richter	101-14Mgt	1103Paulsen	110Richter	1017A	101-14Mgt	Ramsey	Dog Ridge	
1017A	1017A	1017A	1103Paulsen	1017A	140Ruggeri	1023B	420A	USDA 10-17A	USDA 6-19B	
1023B	1023B	1023B	1017A	1023B	1616Couderc	1616Couderc	44-53Malegue	USDA 10-23B	VR O39-16	
1613Couderc	1613Couderc	1613Couderc	1023B	1613Couderc	420A	Dog Ridge	Borner	USDA 6-19B		
161-49	1616Couderc	6-19B	140Ruggeri	Dog Ridge	Borner	K51-32	RS-3			
3306C	34EM	Dog Ridge	1613Couderc	Freedom	Kober 5BB	K51-40	USDA 10-23B			
					Riparia					
3309C	420A	Freedom	1616Couderc	Harmony	Gloire	Ramsey	USDA 6-19B			
Borner	6-19B	Harmony	3306C	K51-32	RS-3	USDA 10-17A	VR O39-16			
Dog Ridge	Dog Ridge	Kober 5BB	34EM	Ramsey	Ramsey	USDA 10-23B				
					USDA					
Freedom	Freedom	Ramsey	420A	Riparia Gloire	10-17A	USDA 6-19B				
					USDA					
Harmony	Harmony	RS-3	Dog Ridge	RS-3	10-23B					
K51-32	IAC 572-Jales	Ramsey	Harmony	Ramsey						
Kober 5BB	IAC 313- Tropical	Rupestris du Lot	IAC 572-Jales	Rupestris du Lot						
Ramsey	K51-32	Schwarzmann	IAC 313- Tropical	SO4						
Riparia Gloire	Kober 5BB	SO4	Ramsey	USDA 10-17A						
RS-3	Ramsey	Teleki 5C	Ramsey	USDA 10-23B						
Ramsey	RS-3	USDA 10-17A	Salt Creek	USDA 6-19B						
Rupestris du Lot	Ramsey	USDA 10-23B	<i>Vitis</i> <i>rotundifolia</i>	VR O39-16						
Schwarzmann	Salt Creek	USDA 6-19B								
SO4	SO4	<i>Vitis rotundifolia</i>								
Teleki 5C	USDA 10-17A									
USDA 10-17A	USDA 10-23B									
USDA 10-23B	USDA 6-19B									
USDA 6-19B	<i>Vitis rotundifolia</i>									
<i>Vitis</i> <i>rotundifolia</i>										
VR O39-16										

long fallow periods that will reduce nematode populations are often uneconomical for growers.

There are post-plant nematode management methods that have been evaluated and reported. Cover crops in established vineyards have shown some nematode control benefits. For example, *Brassica napus* cv. AV Jade and *Brassica juncea* cv. Caliente 199 reduced *M. xenoplax* (Kruiger *et al.*, 2015). Organic amendments also have been shown to give some degree of nematode control. Rahman *et al.* (2014) reported that poultry litter biochar reduced densities of *T. semipenetrans* and *M. xenoplax*. Furthermore, orange oil extract and jojoba oil reduced *M. incognita* in soil and in roots and enhanced the yield of Thompson Seedless vines (Mervat *et al.*, 2012). In another study, jojoba oil also reduced *M. incognita* densities and increased grape yield of the cv. Superior (Ismail *et al.*, 2011). Biofumigation and organic amendments led to a reduction in *Meloidogyne* spp. gall index and to increased yield (Rodriguez *et al.*, 2011). *M. javanica* levels also were reduced using Indian mustard as a green manure and as a seed meal on grape cv. Semillon (Rahman *et al.*, 2011). Dual applications of sweet marjoram extract were effective in a field trial in reducing *M. incognita* and in increasing yield on table grapes cv. Bez-Al-Anza (El-Nagdi and Youssef, 2009). Cattle and chicken manure also showed potential in reducing *M. incognita* under field conditions on Thompson Seedless grapes (El-Sheikh *et al.*, 2006). In two separate vineyards of different age, mustard green manure reduced *M. javanica* (Rahman and Somers, 2005), and in a 6-year-old Cabernet Sauvignon vineyard, growing and incorporating *Cosmos bipinnatus* Cav. provided significant *X. americanum* control (Aballay *et al.*, 2001). A limiting factor on the locally derived amendments is that they can differ greatly in composition and activity from one region to another. Commercially available composts have been found to have more uniformity, and some have been shown to reduce *M. incognita* on grapes cv. Superior (Abd-El-Khair *et al.*, 2009).

Biological control of nematodes in grape has been investigated. For example, rhizobacteria reduced the damage and reproduction of *M. ethiopica* on potted Chardonnay grapes (Aballay *et al.*, 2013). *X. index* numbers also were reduced on Thompson Seedless grapes following treatment with several rhizobacteria isolated from grapevines (Aballay *et al.*, 2012). Grapes inoculated with arbuscular mycorrhizal fungus were shown to

cause local and systemic-induced reductions in *X. index* (Hao *et al.*, 2012). *Pseudomonas fluorescens* added together with farmyard manure reduced *M. incognita* soil populations by 57% over the controls and the gall index in a field trial on grapevines cv. Muscat Hamburg (Senthilkumar and Rajendran, 2004).

In many grape-growing regions, the use of non-fumigant nematicides is permitted. Cadusafos, ethoprophos and carbofuran applied through the drip have shown nematocidal activity towards *M. xenoplax*, *X. index* and *X. americanum* in the table grape Red Globe on its own roots (Magunacelaya *et al.*, 2011). However, there is a tendency to move away from carbamates and organophosphates, and new non-fumigant molecules with less environmental impact are now being investigated. Spirotetramat is a systemic insecticide that can be transported in the phloem and xylem (Brück *et al.*, 2009) and which inactivates juvenile nematode development (Vang *et al.*, 2016). The chemical is registered in California for nematode management in grapes and other crops. McKenry *et al.* (2011) reported that foliar sprays with spirotetramat mixed with an adjuvant controlled *M. incognita* for 6 months, and that nematode reduction was correlated with yield increase of Ruby Seedless grape. In addition, *X. index*, *X. americanum*, *M. xenoplax* and *T. semipenetrans* can also be managed with spirotetramat (McKenry *et al.*, 2009). In grape production, the development of novel synthetic and biological nematode management strategies with lower environmental impact than traditional organophosphates, carbamates and fumigants is currently of utmost importance.

Kiwi

A large number of plant parasitic nematodes have been reported infecting kiwi (*Actinidia chinensis*) worldwide (Table 13.3). However, widespread nematode damage has only been reported for root knot nematodes. In Turkey, *M. incognita* populations in kiwi orchards peak during the month of March (Akyazi and Felek, 2013), which is the optimum time of the year to analyse for nematode infestation. In France and Italy, *M. hapla* and *M. arenaria* induce small, discrete root galls whose histopathology is similar to that on other crops. In both countries, root knot infestations were associated with unthrifty plants.

Table 13.3. Plant parasitic nematodes associated with kiwi listed by country.

Plant parasitic nematodes	Country
<i>Meloidogyne arenaria</i> , ^a <i>Meloidogyne ethiopica</i> , <i>Meloidogyne hapla</i> , <i>Meloidogyne incognita</i> and <i>Meloidogyne javanica</i>	Brazil
<i>M. arenaria</i> , <i>M. ethiopica</i> , <i>M. hapla</i> , <i>M. incognita</i> ^a and <i>M. javanica</i>	Chile
<i>M. ethiopica</i>	Greece
<i>Aphelenchus</i> sp., <i>Aphelenchoides</i> sp., <i>Basirolaimus indicus</i> , <i>Helicotylenchus dihystra</i> , <i>Helicotylenchus indicus</i> , <i>Helicotylenchus kashmiriensis</i> , <i>Longidorus</i> spp., <i>M. hapla</i> , <i>Merlinius</i> sp., <i>Nothotylenchus</i> sp., <i>Pratylenchus penetrans</i> , <i>Psilenchus</i> sp., <i>Sakia typica</i> , <i>Tylenchorhynchus elegans</i> , <i>Tylenchorhynchus oryzae</i> , <i>Tylenchus filiformis</i> and <i>Xiphinema basiri</i>	India
<i>M. incognita</i>	Turkey

Note: ^aHighest frequency found

M. hapla and *M. incognita* are both widespread in kiwi orchards in Chile (Philippi *et al.*, 1996). Although kiwi cv. Hayward seedlings were relatively tolerant to *M. hapla* in pot trials in Chile (Philippi and Budge, 1992), Di Vito *et al.* (1988) found that *M. incognita* race 1 caused serious growth reduction in the same cultivar, with an estimated tolerance limit of 0.43 eggs and juveniles/ml of soil. Contaminated nursery rootstocks can result in serious infestation by *Meloidogyne* spp. in kiwi orchards. The only other record of damage by species other than root knot nematodes is from a pot experiment in which *P. penetrans* reduced kiwi seedling growth (Vrain, 1993). The possibility of interactions between nematodes and major soil-borne pathogens of kiwi fruit such as *Agrobacterium tumefaciens* and *Phytophthora cinnamomi* has been suggested.

There are no reports of resistant rootstocks for kiwi. Chemical bare root stock with ethoprop and fenamiphos can provide acceptable control of root knot infestations in nursery stock. In California, kiwi growers are advised to avoid the use of cover crops that are hosts of root knot nematodes, and to increase irrigation frequency in infested orchards (McKenry, 2016). Pre-plant fumigation with 1,3-dichloropropene or metam sodium can also be used as a nematode management tool if economically feasible. Reports of nematode management in kiwi in Europe and elsewhere have focused on organic farming methods. Mycorrhizal kiwi were shown to be more tolerant of *M. javanica* than non-mycorrhizal seedlings (Verdejo *et al.*, 1990), and several studies report some control of root knot nematodes with various organic amendments, mulches and biological control agents (Cayrol *et al.*, 1991; Gonzalez, 1993; Maccari *et al.*, 1993).

Passionfruit

Although a number of plant parasitic nematodes are reported associated with passionfruit (*Passiflora edulis*), such as *H. dihystra*, *Mesocriconema* spp., *H. strictathecatus*, *Paratylenchus* spp., *Tylenchorhynchus annulatus* and *X. brevicolle* (Castellano *et al.*, 2012), only reniform and root knot nematodes are reported to cause economic damage. Both nematodes can severely limit fruit production and plant longevity.

R. reniformis is reported to enhance collar rot, and plant life is doubled when infested soil is treated with fenamiphos prior to planting. The reniform nematode is associated with passionfruit in several countries, including Belize, Colombia and Brazil (Sanchez *et al.*, 1993; Bridge *et al.*, 1996; Sharma *et al.*, 2000). Little is known about cultivar susceptibility. Ten cultivars of *P. edulis* were all hosts of a Brazilian population of *R. reniformis*; however, the nematode did not reduce the growth of any cultivar and stimulated growth of the majority (Sharma *et al.*, 2001).

M. incognita, *M. javanica* and other root knot nematodes appear to vary in pathogenicity to passionfruit. In Kenya, it has been suggested that root knot nematodes are not an economic problem on the crop (Ondieki, 1975). In Fiji, *M. incognita*, *M. arenaria* and *M. javanica* did not reproduce on the yellow variety of passionfruit or affect plant growth in pot studies (Kirby, 1978). Therefore, passionfruit is recommended as a suitable rotation crop in Fiji against root knot nematodes. Significant resistance based on root galling was also reported for both the yellow and purple variety of passionfruit toward *M. incognita* and *M. javanica* in

Brazil (Klein *et al.*, 1984; Da Costa *et al.*, 1997; Sharma *et al.*, 2002). In South Africa, however, *M. javanica* and possibly other species are considered as serious pests on yellow, and especially purple, passionfruit (Milne, 1982a). It is unclear whether damage is due to initial penetration intolerance of seedling and young plant roots by the nematode or to resistance to parasitism. The use of rootstocks such as *Passiflora caerulea*, which are tolerant to root knot nematodes, has been suggested as a management tool (Milne, 1982a; Terblanche *et al.*, 1986). Since the passionfruit is relatively short-lived and seedling establishment is of great importance, crop rotations should also be useful for nematode control (Milne, 1982a). Passionfruit has also been suggested as a good rotation crop in South Africa against *R. similis*, which does not infect either *P. edulis* or *P. edulis* f. *flavicarpa* (Milne and Keetch, 1976).

Miscellaneous Fruit Trees

Acerola

Acerola (*Malpighia glabra*) can be severely damaged as a result of *M. incognita* infection (Fig. 13.7). *M. incognita*, *M. arenaria* and *Rhizopus nigricans* (*Rhizopus stolonifer*) are considered the major limiting factors to acerola production in Brazil (Holanda *et al.*, 1997). A report has cautioned that *M. incognita* races 1, 2, 3 and 4, *M. javanica* and *M. arenaria* race 2 can be found on acerola nursery stock (Da Costa *et al.*, 1999). Interestingly, some *Malpighia* species are hosts of *R. reniformis*;

however, some cultivars can be resistant to *R. reniformis*, *R. similis*, *T. semipenetrans* and *Meloidogyne graminicola*. Root knot nematodes are also recognized as economic pests of acerola in Hawaii, and especially in Florida. Fenamiphos treatment was found ineffective for nematode control (McSorley and Parrado, 1982). Several clones of *Malpighia emarginata* were resistant to *M. javanica* in Brazil (Gomes *et al.*, 2000).

Breadfruit

Two important nematodes, the root knot nematode *Meloidogyne* spp. and the reniform nematode, *R. reniformis*, have been reported to attack breadfruit (*Artocarpus altilis*) (Sharma and Sher, 1973; Razak, 1978; McSorley, 1992). Coates-Beckford and Pereira (1992) found high densities of *P. coffeae*, along with *M. incognita* and *Helicotylenchus* spp., in the roots and rhizospheres of breadfruit in Jamaica, but tree health appeared to be related more to tree age than to nematode density.

Loquat

Despite its considerable economic importance, nematodes affecting loquat (*Eriobotrya japonica*) cultivation have not been studied. Perhaps the only two potentially pathogenic nematodes known to attack loquat are *R. macrodorum*, which was found to reproduce and induce histological



Fig. 13.7. Acerola roots infested with *Meloidogyne javanica* from Bahia, Brazil. (Photograph courtesy of R. Ritzinger.)

changes in loquat roots (Inserra and Vovlas, 1980), and *P. vulnus*, which reproduced on loquat cv. Nadal (Pinochet *et al.*, 1992).

Mangosteen

Little is known about nematode problems affecting mangosteen (*Garcinia mangostana*). It is noteworthy that mangosteen has been reported from India as a host of the citrus nematode, *T. semipenetrans* (Chawla *et al.*, 1980).

Pomegranate

A variety of plant parasitic nematodes can be found in pomegranate (*Punica granatum*) orchards, such as *Xiphinema insigne* and *X. brevicolle* in China, *P. neglectus*, *Merlinius* species and others in Iran, and *M. xenoplax* and *Tylenchorhynchus* spp. in California. *M. javanica* and *M. enterolobii*.

Non-fumigant nematicides have been common tools used for nematode control. For example, fenamiphos application gave good control of the root knot nematodes and provided protection to roots for 60 days against nematode invasion and improved fruit yields (Siddiqui and Khan, 1986). Treatment of pomegranate with carbofuran reduced populations of *M. incognita*, *X. insigne* and *Helicotylenchus* spp., and increased yields by one-third (Darekar *et al.*, 1989). Among 23 nematode species found in the rhizosphere of pomegranate in Jordan, Hashim (1983a) reported particularly large populations of *Helicotylenchus pseudorobustus*, *Tylenchorhynchus clarus* and *Longidorus* sp. associated with trees showing severe decline symptoms. However, the application of carbofuran did not improve tree performance. In a sandy soil orchard in China, the efficacy of isazofos was 81.5% against *M. incognita*, and was more effective than ethoprophos and carbosulfan (Liu, 2007). Recently, options other than non-fumigant nematicides have been studied. Mustard and castor oil were evaluated under field conditions and both reduced plant parasitic nematode populations, although efficacy was not as high as carbofuran (Aly *et al.*, 2011). However, there were no differences in yield among treatments. Combining organic amendments with biocontrol agents was reported to have a very high root

knot nematode control efficacy. Castor oil and *Purpureocillium lilacinum* combined treatment was more effective in controlling root knot nematodes than the oil combined with *Pochonia chlamydosporia* or *P. fluorescens* (Somasekhara *et al.*, 2012). No root knot resistant cultivars have been reported.

Sapodilla

Nematodes affecting sapodilla (*Manilkara zapota*) were investigated by Saeed (1974), who demonstrated the pathogenicity of *H. mangiferae* to sapodilla at a population density of 6 nematodes/ml of soil. That study reported population build-up of *Helicotylenchus indicus* and *Pratylenchus* spp. around sapodilla roots. Seasonality of *H. mangiferae* on the crop in India and Pakistan coincides with rainfall patterns (Ashokkumar *et al.*, 1991). *H. dihystrera*, *Mesocriconema* spp., *H. strictathecatus*, *Paratylenchus* spp., *T. annulatus*, *X. brevicolle*, *Aphelenchus* spp., *Aphelenchoides* spp., *Tylenchus* spp., and *Psilenchus* spp. were reported to be associated with sapodilla in Venezuela (Castellano *et al.*, 2012).

Soursop

Soursop (*Annona muricata*) can be a suitable host for several *Helicotylenchus* species, including *Helicotylenchus cavenessi*. *P. coffeae* has been shown to be the causal agent of 'sudden death' of soursop in Brazil (De Moura *et al.*, 1998), and isolates of *P. coffeae* collected from soursop or from yam were capable of causing the disease (De Moura *et al.*, 1999). Control of *X. ifacolum* and *H. pseudorobustus* with carbofuran did not increase the growth of soursop in nurseries in Liberia (Lamberti *et al.*, 1992).

Tamarind

Of the several nematode species associated with tamarind (*Tamarindus indica*), only *H. mangiferae* has been considered as pathogenic at a population density of 6 nematodes/ml of soil (Saeed, 1974). The tamarind has also been reported as a host of *R. similis* (Sosamma and Koshy, 1977).

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14 Nematode Parasites of Coconut and other Palms*

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The botanical order Arecales has but a single family, Arecaceae, also known as Palmae. Palm is the common name for any flowering plant of the family. Although many of the 2800 known species of palms have some particular economic importance to any given local population, only a few are of major economic importance worldwide.

Cocos nucifera, the coconut palm, which originated in the Old World tropics, is now widely distributed throughout the tropics.

Elaeis guineensis, the African oil palm, with its origin in Central Africa, has now been introduced throughout the tropics, including Latin America.

Phoenix dactylifera, date palm, is native to the Near East, where it has been cultivated for its fruit for nearly 8000 years.

Areca catechu, arecanut, occurs mainly in the humid regions of Asia and the Malay Islands. It was introduced into India in the pre-Christian era, where it is now widely cultivated and used as a masticatory.

Metroxylon spp., the sago palms, provide a starchy food material that is stored in their

trunks as they develop to the point of flowering. These palms are hypoxanthic and only mature palms just prior to flowering and death are cut and used for starch production. These palms are also used during and after starch production for palm weevil (*Rhynchophorus bilineatus*) larval culture for human consumption in Papua New Guinea (Giblin-Davis, 2001). Sago palms of this genus are native to the Indonesian archipelago.

The fruit and seeds of eight genera of the world's palms are oil bearing and can be exploited commercially for oil. Only *Cocos* is entirely of an Old World origin; *Elaeis* has one species (*guineensis*) that is of Old World origin and another (*oleifera*) that belongs to tropical America. The other six genera are considered neotropical. There are many palms that are ornamental and are important in horticulture and landscaping.

Coconut

Recent molecular evidence suggests that an Indian Ocean derived coconut line ('typical' form)

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was moved to the west coast of Africa by the Portuguese, and then into the Caribbean and coastal Brazil (Gunn *et al.*, 2011), whereas a Pacific Ocean derived lineage of coconut was apparently moved by Austronesians in pre-Colombian times during eastern incursions into the Pacific coastal neotropics (e.g. Panama), and later by the Spanish through the movement of coconuts from plantations in the Philippines to the Atlantic coast of Mexico (Gunn *et al.*, 2011).

Coconut palm is most adapted to temperatures around 27°C with a diurnal range of about 7°C; it does not thrive at temperatures lower than 20°C and is damaged at temperatures below 15°C. Rainfall requirements are about 2500 mm/year; when less than 1000 mm/year, irrigation is normally necessary. The absence of rain for more than 3 successive months causes a shedding of young fruit and a reduction in fruit size. The palm does best with about 2000 h of sunlight/year, or an average of 6 h/day. Thus, with few exceptions, cultivation is limited by the 20° parallels of latitude and the 500 m contour line.

The largest producers of coconuts are Indonesia, the Philippines, India and Sri Lanka. Significant quantities are produced, however, from most tropical countries of South and East Asia, East and West Africa and the Pacific. Despite there being some large plantations, coconuts are predominantly a smallholder crop, with about 96% of the crop being produced by over 10 million poor smallholders with average land holdings of less than 1 ha.

The total world area under coconuts was estimated in 1996 as being over 11 Mha, with a total production of over 47 metric tonnes (Mt) (FAO, 2003). The two biggest producers, Indonesia and the Philippines, had about 3.7 and 3.1 Mha, respectively; India was the third largest producer, with nearly 1.8 Mha under cultivation. In all producing countries, coconuts make a significant contribution to the diet, in addition to being an important source of export earnings.

Nematodes of Coconut

Many different nematodes have been found in diverse forms of association with the living coconut palm. Others have been found associated in different types of symbiosis with insect visitors of the palm, operating and existing in various

niches (see references in Griffith *et al.*, 2005), but the major nematode disease affecting the crop is red ring disease caused by *Bursaphelenchus cocophilus*. The only other nematode known to cause severe damage leading to disease in the coconut is *Radopholus similis*.

Bursaphelenchus cocophilus

The red ring nematode, *B. cocophilus*, was first described by Cobb (1919) as *Aphelenchus cocophilus* from specimens sent from Grenada. Since then, it has been known as *A. (Chitinoaphelenchus) cocophilus* and *Chitinoaphelenchus cocophilus* (Micoletzky, 1922), *Aphelenchoides cocophilus* (Goodey, 1933) and *Rhadinaphelenchus cocophilus* (Goodey, 1960). Giblin-Davis *et al.* (1989b) presented morphological evidence supporting the similarities between *Rhadinaphelenchus* and *Bursaphelenchus*. Baujard (1989) synonymized the monotypic genus *Rhadinaphelenchus* with *Bursaphelenchus*, creating the new combination, *B. cocophilus*. Hunt (1993) retained the genus *Rhadinaphelenchus* on the grounds of some typological differences and history, but recent molecular inferences using the 18S SSU rRNA (small subunit ribosomal ribonucleic acid) gene, D2/D3 expansion segments of the LSU (large subunit) rRNA gene, ITS-1/2 (internal transcribed spacer) rRNA and a partial sequence of COI (cytochrome oxidase I) mitochondrial DNA support the inclusion of *B. cocophilus* within the *Bursaphelenchus* radiation (clade 4) with *Bursaphelenchus platzeri* as a putative sister species (Ye *et al.*, 2007; Kanzaki and Giblin-Davis, 2012, 2016; Silva *et al.*, 2016).

Brief history of red ring disease

The disease was first reported as occurring in Trinidad by Hart (1905). The first investigations into its nature were by Stockdale (1906), who thought that two different diseases were being confused because they both culminated in decay of the bud. One of these was red ring disease, then called root disease, and the other bud rot initiated by *Phytophthora palmivora*. Barrett (1906), however, reported that there were few genuine cases of bud rot among the coconuts in Trinidad, and that 95% of the losses were really due to root disease.

Nowell (1919) found that a large number of roots examined from diseased trees in Trinidad contained hundreds of nematodes of the same species. They were also present in the constant red ring found in trees in Grenada and also in the material collected in Trinidad by Rorer (1911). Later, he examined stained sections from many other sources and confirmed Rorer's earlier conclusion that a fungus was not the causal organism, but noted that nematodes of the same species previously observed were constantly present in stems, leaves and roots. The name red ring disease was then used by Nowell (1919) and became established.

Distribution of red ring disease

At present, red ring disease has a restricted distribution in tropical America and has only been reported from the West Indies (Trinidad, Tobago, Grenada and St Vincent) and from Latin America (Venezuela, Guyana, Surinam, French Guyana, Colombia, Ecuador, Peru, Mexico, Brazil, Panama, Nicaragua, Costa Rica, Honduras, Belize and El Salvador). It is also reported that red ring disease occurs in Guatemala, but does not occur in the northern Caribbean islands, Florida, Cuba or other parts of the world (Dean, 1979). There are unconfirmed reports of red ring disease from Barbados, Dominica and Jamaica, but the European and Mediterranean Plant Protection Organization (EPPO) considers the disease to be absent from these countries. Some sources have reported the nematode present in the Bahamas, Dominican Republic and Haiti; however, we consider these questionable assertions. A single diseased palm was discovered in Dominica in 1982, but after its destruction, there has been no further incidence of the disease in that country. The disease in Barbados claimed to be red ring in 1995 was found to be Cedros wilt, a disease caused by a protozoan flagellate, *Phytomonas stahellii* (R. Griffith, unpublished). CAB International stated in their Crop Protection Compendium data sheet that there were unconfirmed reports of the nematode being present in Puerto Rico in 2002; however, CAB International now record it as 'Absent, unreliable record' from the country. None of the more recent surveys has found the nematode in Puerto Rico (CAB International, 2018).

Symptoms of red ring disease

Young or adolescent coconut palms succumb easily to red ring disease. There is no record of

any tree, once affected, having recovered. The disease occurs more commonly in trees 2.5–10 years old, with the greatest incidence in those 4–7 years old. Occasionally, a palm as young as 1.5 years or as old as 20 years or more may be attacked.

The symptoms characteristically described are those for palms of the tall cultivar of coconuts or 'typical' form from the Indian Ocean derived lineage of coconut (associated with the more ancestral 'niu kafa' form of oblong and triangular fruit, with a relatively high proportion of husk fibre that is adapted for ocean dispersal) (Gunn *et al.*, 2011). These symptoms differ somewhat in the dwarf coconut cultivar called 'nana' (associated with the human-selected 'niu vai' fruit morphology, with rounded, brightly coloured nuts and relatively abundant endosperm) (Gunn *et al.*, 2011), and also some 'Panama tall'. Chlorosis first appears at the tips of the oldest leaves and spreads towards their bases, but occasionally, one of the younger leaves may be affected first. The brown lower leaves may break across the petiole or the lower part of the rachis, or they may become partly dislodged at the base and hang down (Fig. 14.1). Nuts are shed prematurely, either simultaneously with the development of leaf symptoms or slightly before. The crown often topples over about 4–6 weeks after the symptoms first appear, due to associated severe damage caused internally by the larvae of the palm weevil. However, the trunk remains standing in the field for several months, until it decays. At the onset of symptoms, the chlorotic yellow appearance of the leaves around the stem is sometimes indistinguishable from that of trees growing under conditions of poor drainage or during intense drought.

The most characteristic symptoms are the internal lesions. In a cross section of the stem, they appear as an orange to brick-red coloured ring, 2–4 cm wide, and at a distance of 3–5 cm in from the periphery (Figs 14.1 and 14.2). In longitudinal section, the reddened tissue may appear as two united bands joined in the bole, forming a 'U' shape (Fig. 14.2). Lesions at the upper end of the stem in the vicinity of the crown are discrete, appearing first as streaks and then as dots. The meristematic tissue in the bud remains white and apparently healthy. Occasionally, in some older trees, the entire central cylinder of the stem becomes one solid block of



Fig. 14.1. (a) Characteristic red ring symptoms in a dying coconut palm in Trinidad. (b) Cross section of coconut stem infected by *Bursaphelenchus cocophilus*. (Photographs courtesy of R. Giblin-Davis.)

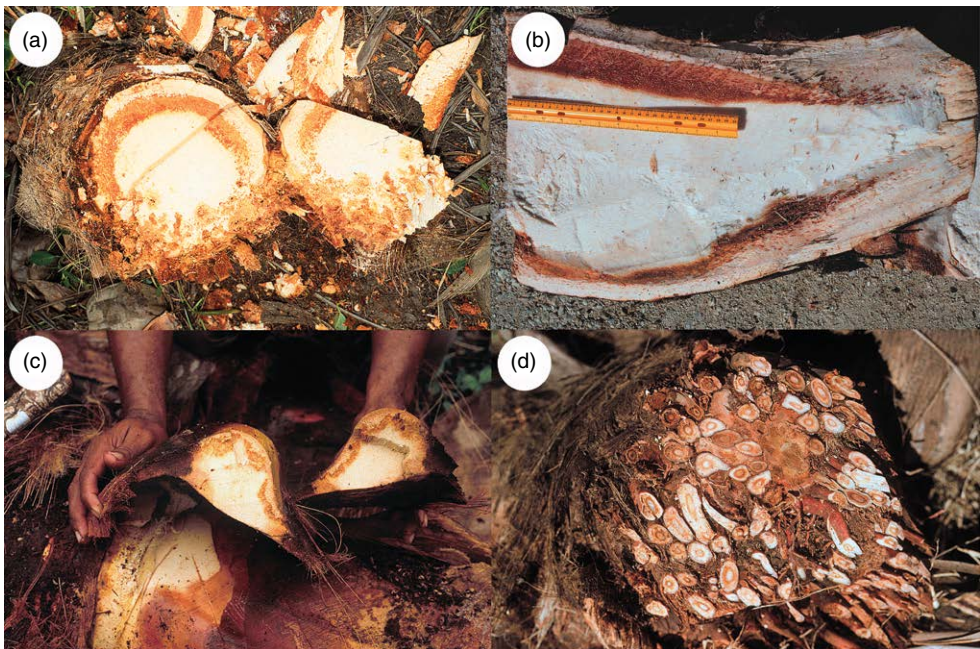


Fig. 14.2. (a) Cross section of coconut stem infected by *Bursaphelenchus cocophilus* with *Rhynchophorus palmarum* larval tunnelling damage along bottom edge. (b) Longitudinal section of coconut stem showing red ring tissues in two united bands joined in the bole, forming a 'U' shape. (c) Red ring symptoms in cross sections of coconut petioles. (d) Red ring symptoms in cross sections of coconut roots. (Photographs courtesy of R. Giblin-Davis.)

red. There is no putrefaction of the bud associated with red ring disease. In the roots, the normally white soft cortex becomes orange to faint red in colour, and dry and flaky in texture when diseased. In the leaves, a solid core of mottled tissue, dull red to brown in colour, extends from the leaf base for varying distances up to about 75 cm in the petioles (Fig. 14.2).

In its very early stages, the disease is not recognizable externally. The roots, stems and leaf petioles are already infested and there is full development of internal symptoms before the first external symptoms become visible. In the dwarf cultivars, the red colour gives way to shades of brown. Thus, instead of a red ring internally, there is a brownish band. The discrete spots are also brownish and the yellow discoloration of the leaves is often not apparent. Generally, the leaves become dried and brown, beginning at the tips of the leaflets and progressing downwards. The yellow dwarf cultivars respond

in the same way as the green, and the crosses between tall and dwarfs or between 'Panama tall' and any dwarf. They show a browning instead of a characteristic reddening of the leaves and stem tissue.

Biology of the red ring nematode

The chief vector of the red ring nematode is an insect, the palm weevil (*Rhynchophorus palmarum*), and the biology and life cycle of *B. cocophilus* are intimately associated with this and other palm-associated weevils, such as *Dynamis borassi* and *Metamasius hemipterus* (Giblin-Davis, 2001) (Figs 14.3 and 14.4). However, experimentally, it has also been shown that red ring disease can be initiated by the nematodes via the root system through soil.

Studies on the biology of the nematode were initiated around 1919 by both Cobb and Nowell. Cobb found that 50% of adult *R. palmarum* and

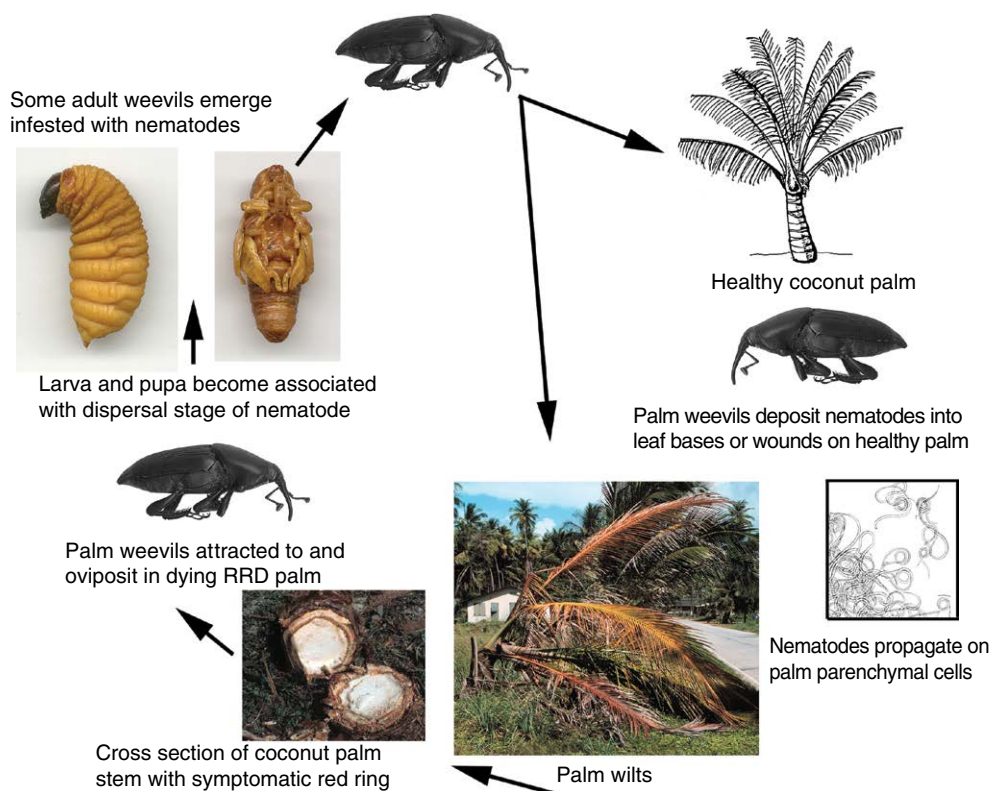


Fig. 14.3. Life cycle of the coconut palm weevil *Rhynchophorus palmarum* and transmission of the red ring nematode *Bursaphelenchus cocophilus*. (R. Giblin-Davis.)

Note: RRD palm = red ring diseased palm.

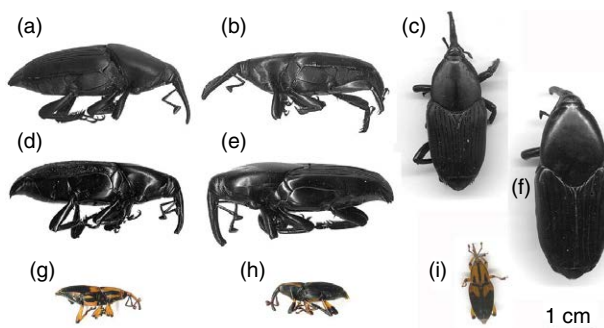


Fig. 14.4. Palm and sugarcane weevils known or suspected to be vectors of the red ring nematode. (a and b) *Rhynchophorus palmarum* female and male, respectively; (c) male dorsal aspect; (d and e) *Dynamis borassi* female and male; (f) male dorsal aspect; (g and h) *Metamasius hemipterus* female and male; (i) female dorsal aspect. (R. Giblin-Davis.)

their larvae contained the red ring nematode. As a result, he implicated the palm weevil as a carrier of the nematode from diseased palms to healthy ones. Contrastingly, Nowell's opinion was that the nematode was soil inhabiting.

The general consensus is that *B. cocophilus* does not build up large populations in the soil, as some of the earlier investigators had believed to support their early recommendations of isolated trenches to control the movement of the nematode (Martyn, 1953). It would seem, moreover, that although root infection could be induced artificially, it was not a normal method of initiation of the disease in the field. In the ordinary course of events, the nematodes would not persist in the soil in sufficient numbers to give a reasonable chance of infection, unless the roots of an infected tree overlapped with an uninfected tree, or if a new seedling was transplanted into soil where a palm had recently died from red ring disease. If a persistent source of inoculum was present, for example a buried red ring trunk (which remains quite fresh for 2 weeks after burial), or if a high population of *B. cocophilus* (10^4 nematodes/cm³) was added artificially to the soil in large quantities of water, the nematodes could gain entry through damaged or senescent roots and eventually migrate up into the trunk, producing the usual symptoms.

Transmission of red ring nematode

Larvae of the palm weevil *R. palmarum* feed by burrowing through coconut stems, and when

this occurs in trees with red ring disease, they can become infested with the nematode. Adult weevils emerging from diseased trees carry the nematode to new sites (Figs 14.3 and 14.4). Nematodes enter the haemocoel of weevil larvae via the gut tract; in newly emerged adult weevils, the nematodes can be found in the tracheae, gut, body cavity and the region of the ovipositor (Griffith, 1968a).

Survival of the red ring nematode depends on the third-stage juvenile. They are sometimes found in tracheal sacs in the insect, from where they can move directly to the ovipositor of the female vector palm weevil (Griffith, 1968a). The percentage of palm weevils associated with red ring nematodes (up to 100%) and the levels of infestation (>13,000 nematodes/insect) can be quite variable. Evidence has been presented that indicates a connection between weevil size and percentage of weevils carrying the nematodes (Griffith, 1968a). However, other results have shown that variation in the levels of infestation apparently are not correlated with other variables, such as weevil size (Giblin-Davis, 1993). The nematodes are putatively injected into the tissues of the coconut tree when the insect deposits its eggs, normally in a leaf axil in the crown of the tree (Griffith, 1968a,b) (Fig. 14.3). The palm weevil might be considered an obligate transportation host, whereas the coconut palm, in which the nematode multiplies, could be considered as the definitive host.

The palm weevil *D. borassi* (Fig. 14.4) can carry close to 2000 red ring nematodes

through metamorphosis and is assumed to be a potential vector in coconut, where it is sometimes a pest in South America, for example Ecuador, Colombia and Brazil, in unopened inflorescences and the crown of coconut (Gerber *et al.*, 1990; Giblin-Davis, 2001).

The weevil *M. hemipterus* (Fig. 14.4) has been incriminated as a vector of red ring nematodes in Colombia (Mora *et al.*, 1994), but not in Costa Rica (Bulgarelli *et al.*, 1998) or Trinidad (Hagley, 1963). Trap captures of *M. hemipterus* in African oil palm plantations tended to be up to 35 times higher than *R. palmarum*, but the percentage association and numbers of red ring nematode per weevil were lower with *M. hemipterus* than with *R. palmarum* (Mora *et al.*, 1994). *M. hemipterus* may become associated with red ring disease because it attacks the pruned or damaged petioles and frond bases of living coconut, African oil palm, date and other palms (Giblin-Davis, 2001), which are development sites for the red ring nematode (Giblin-Davis *et al.*, 1989a).

Palm and sugarcane weevils in the Dryophthoridae, such as *R. palmarum*, *D. borassi* and *M. hemipterus*, are highly attracted to the volatiles emanating from recently wounded or pruned palms, red ring diseased palms, and moist fermenting tissue from palms, sugarcane stalks, various fruit and molasses (Chittenden, 1902; Giblin-Davis, 2001). In addition, males of these weevils produce male- and female-attracting aggregation pheromones identified as methyl-branched secondary alcohols (Giblin-Davis *et al.*, 1996; Giblin-Davis, 2001). The main aggregation pheromones are (4*S*,2*E*)-6-methyl-2-hepten-4-ol for *R. palmarum* (Rochat *et al.*, 1991; Oehlschlager *et al.*, 1992b), and (4*S*,5*S*)-4-methyl-nonan-5-ol for *D. borassi* (Giblin-Davis *et al.*, 1997) and *M. hemipterus* (Rochat *et al.*, 1993; Pérez *et al.*, 1997), which are commercially available as synthetic racemic blends at ChemTica Internacional SA.¹ These pheromones work as synergists with fermenting palm, sugarcane, or pineapple tissue and can be used to create lethal traps that are effective for monitoring or mass trapping efforts. The addition of the kairomone ethyl acetate² ('weevil magnet') can improve pheromone trap capture efficiency (Giblin-Davis *et al.*, 1996). In addition, *R. palmarum*, *D. borassi* and *M. hemipterus* respond to each others' pheromones, increasing the

chances that once a weevil finds a stressed tree, other weevils will help to overcome it and use it as a host (Giblin-Davis, 2001). This phenomenon may also increase the chances for spreading red ring disease. In nature, the combination of weevil recruitment through chemical ecology and the killing potential of the red ring nematode may function together as a form of populational mutualism where enhanced reproduction of both partners is the result of the association (Giblin-Davis, 2004).

Biology of B. cocophilus in coconut tissues

The nematodes naturally invade only parenchymatous tissue in roots, stems and leaves, and artificially inoculated nuts. At first, nematodes occur as intercellular parasites in newly invaded tissue, but later they can be found both intercellularly and intracellularly. In many cases, lysigenous cavities are formed in which large numbers of nematodes are present. One gram of such tissue can contain as many as 10,000 nematodes. Nematodes have never been found in xylem vessels, nor has there been any evidence of direct damage to the tracheal elements. Despite this, however, many of the vessel elements in the discoloured areas become occluded with tyloses. It has been shown that the uptake of water injected into the stems of trees is much slower in diseased trees than in healthy trees. Thus, one feature of the external symptoms coincides with a pathological condition due to water imbalance in the plant.

The cause of the restriction of nematodes to the narrow band or ring of necrotic tissue in stems has never been explained satisfactorily. Nowell (1923) found no anatomical or physiological factors in trees that might have accounted for it. Martyn (1953) expressed the view that the outer limit of the red zone was determined by the harder tissue at the periphery of the stem, and the inner limit was set by aeration and water supply. Nevertheless, occasionally, there is a solid cylinder of discoloured tissue instead of just a band. Nematodes are often found intercellularly in white, apparently healthy tissue for 1 cm on the outside and 2.5 cm on the inside of the red ring tissue. They are less abundant here than in the body of the ring, where they are found both intercellularly and intracellularly. It would, therefore, appear that there are

other factors that naturally limit the occurrence and activity of the nematode on the outside and inside of the ring. The most outstanding characteristic of all tissue invaded by *B. cocophilus* is the presence of relatively large intercellular spaces. The inadequacy of intercellular space may, therefore, determine the outer limit. The colour of the band appears to be a specific plant chemical reaction to the invasion, and this varies in the tall and dwarf forms of coconut.

Nematodes inoculated into the mesocarp of nuts were found to have a life cycle, from egg to egg, of 9–10 days (Blair, 1964). The red ring nematode can persist in the diseased coconut tissue for about 3 months (Griffith, 1968b). Ashby (1921) found that juveniles were extremely susceptible to desiccation. They died within 6 h of drying and 15 h when provided with small fragments of tissue. The absence of moisture for half an hour only, followed by exposure to a saturated atmosphere for 24 h, resulted in death of the juveniles in nine cases out of ten.

There are other nematode species besides *B. cocophilus* associated with *R. palmarum*, *D. borassi* and *M. hemipterus* (Griffith, 1968a; Gerber and Giblin-Davis, 1990a,b) that might cause some confusion during dissections. However, all of these additional nematode associates, such as *Teratorhabditis palmarum* (Gerber and Giblin-Davis, 1990b), *Acrostichus rhynchophori* (Kanzaki *et al.*, 2009), *Bursaphelenchus gerberae* (Giblin-Davis *et al.*, 2006) and *Caenorhabditis angaria* (= *Rhabditis* sp.) (Sudhaus *et al.*, 2011), have been confirmed to be phoretic saprobionts.

Environmental factors affecting red ring disease

The larvae of the palm weevil often die when they develop in a tree that is attacked by *P. palmivora* (bud rot) or *Micrococcus roseus* subsequent to the contracting of red ring disease. Cannibalism in larvae of the palm weevil resulting from overcrowding often affects the number of emerging weevils. It is known that the red strain of *M. roseus* produces disease and septicaemia in affected palm weevils. Some ground lizards also feed on the adult insects.

The heaviest losses due to red ring disease occur at the end of the wet season and in the first 2 or 3 months of the dry season, i.e. between December and March, in Trinidad (Hagley, 1963).

The abundance of the disease may be associated with pruning activities or with the activities of other insects that wound the tree first, inducing fermentation, to which the palm weevil is attracted for oviposition.

The palm weevil is a pest in its own right and may relate to the environment differently. *R. palmarum* is a pest of the coconut palm, the gru-gru palm, *Acrocomia aculeata*, and several others. Some of the host palms are wild in the forest and in other uncultivated areas of Latin America, and many represent reservoirs that could become a source of migrant insects. However, epiphytotic in wild palms have not been reported.

In many Latin American countries, there exist different levels of attack from red ring disease only and palm weevil attack without red ring disease. In Ecuador, where the Creole tall palms seem to have more Panama Tall stock, the palm weevil is a major pest and the adult insects attack healthy trees of any age. In other countries, such intense attack without red ring disease is quite rare, but in Ecuador, the insect is a pest in a habitat consisting of several other interplanted kinds of food sources for the weevil, such as pineapples, papayas and sugarcane, which are non-hosts for red ring nematode.

The effects of climate on red ring disease incidence are very apparent as one moves from the dry southern Ceara coconut regions to the northerly, more humid areas such as Bahia in Brazil. In Ceara, where the dry season extends for 7.5 months, the incidence of red ring is significantly lower than in Rio Grande del Norte, where the dry season is for 5.5 months, and less than in Paraiba, where the season lasts for 3.5 months. However, in Pernambuco, where the dry season lasts for only 2 months, the incidence is almost as high as in Bahia Sul, where there is little or no dry season. The larvae of the palm weevil can develop adequately within the tissues of the coconut trunk; however, the dissemination stage of the adult is affected by the low humidity in the driest regions, where one is more likely to find dead adult palm weevils in the field.

Other hosts

Although red ring is primarily a disease of the coconut palm, it has been found in many palms (Table 14.1), including an unidentified species of *Cocos* (probably a *Syagrus* sp.) in the Botanic

Table 14.1. Natural and inoculated host list records for *Bursaphelenchus cocophilus*.

<i>Acrocomia aculeata</i> (gru-gru palm)
<i>Acrocomia intumescens</i>
<i>Attalea cohune</i> (Cohune palm)
<i>Bactris gasipaes</i>
<i>Bactris</i> sp.
<i>Cocos nucifera</i> (coconut)
<i>Cocos</i> sp.
<i>Elaeis guineensis</i> (African oil palm)
<i>Euterpe pacifica</i> (<i>E. precatoria</i> , or <i>Mauritiella pacifica</i>)
<i>Jessenia polycarpa</i>
<i>Mauritia flexuosa</i> (Ita palm)
<i>Mauritia caribea</i> (Cocorite)
<i>Mauritia mexicana</i>
<i>Maximiliana maripa</i>
<i>Oenocarpus distichus</i>
<i>Phoenix canariensis</i> (Canary Island date palm)
<i>Phoenix dactylifera</i> (date palm)
<i>Roystonea oleracea</i> (royal or cabbage palm)
<i>Roystonea regia</i>
<i>Sabal palmetto</i> (Sabal palmetto)
<i>Sabal</i> sp.

Gardens, Grenada (Nowell, 1924), and the date palm, *P. dactylifera*, in the Botanic Gardens, Trinidad. Hagley (1963) found one case of natural infestation of the cabbage palm *Roystonea oleracea*. Disease incidence was reported to be high in the plantation of oil palms, *E. guineensis*, in Venezuela in 1953 (Malaguti, 1953). Nowell (1924) reported successful inoculation of the cabbage and the gru-gru palm. Various ornamentals have been inoculated artificially, among them are the Sabal palm, *Sabal palmetto*, and the cocorite palm *Mauritia caribea*. The disease has also been found in Brazil on *Attalea cohune*, the Cohune nut.

Criconemoides curvatus

The palm weevil does not transmit the nematode to any other non-palm host species, e.g. sugarcane, papaya or pineapple. However, countries such as the USA, which use *S. palmetto* for decorative purposes in the presence of the American palmetto weevil, *Rhynchophorus cruentatus*, need to ensure proper surveillance or eradication measures against both the palm weevil *R. palmarum*, which is considered a transient species in southern California, Texas and Arizona (Hodel

et al., 2016), and the red ring nematode, which has not been detected (Esparza-Diaz *et al.*, 2013). The date palm, the coconut and the sabal palm are all suitable hosts for *R. cruentatus*, which could be a suitable alternate vector for the red ring nematode, if the opportunity for acquisition arises (Giblin-Davis *et al.*, 2013).

Epidemiology and general management measures

Red ring disease in new groves generally begins by infection of a 4- to 10-year-old palm by a weevil carrying the nematodes. The most effective management should be implemented during the initial phase of palm weevil and red ring disease infestation to prevent the development of an epiphytotic.

The rate of spread from a primary infector plant depends on the development of vector palm weevils within the diseased tree. Typically, 3 months after infection, nearby susceptible healthy trees can be infected by a vector(s) emerging from the infector plant (Fig. 14.3). The initially diseased tree remains a source of red ring nematode inoculum for 6–8 weeks after its death as it continues to attract other sugarcane and palm weevils that might become contaminated with red ring nematodes and serve as vectors. Phytosanitary measures of control are critical at this time, since disease symptoms are apparent before the vector progeny emerge, and successful intervention can prevent an epiphytotic of red ring disease.

Emerging palm weevils disperse to the leaf axils of diseased trees or wounded trees emitting attractive compounds (kairomones such as ethyl acetate and ethanol) (Giblin-Davis *et al.*, 1996; Roach *et al.*, 2000). In addition, the males of *R. palmarum*, *D. borassi* and *M. hemipterus* produce aggregation pheromones that synergize attraction and recruit conspecifics and heterospecifics of both sexes (Giblin-Davis, 2001). These weevils oviposit in newly diseased palms and cause increased insect populations. Control measures relate directly to the abundance of the disease. Since all red ring diseased trees are breeding grounds for palm and sugarcane weevils and red ring nematodes, the destruction and removal of these trees and the reduction of their attractiveness is essential to preventing epiphytotics.

Specific management measures for red ring disease in coconut

There are no simple means of controlling red ring disease, and no effective measures are available as yet for control of the nematode in living palms. Control is based on prevention rather than cure, by the destruction of infested palm material and by the trapping and killing of the weevil vectors before they spread the nematodes.

Many trees show yellowing and browning of leaves that may not be due to red ring disease. To prevent the unnecessary destruction of trees, a 'core sample' of the trunk should be taken with a 2 cm diameter pipe (see below) to determine the presence of red ring disease and the nematodes before control measures are employed. However, the coring hole needs to be sealed or treated with a pesticide to avoid creating an attractive wound for palm weevil attack.

INSECTICIDE AND HERBICIDE TREATMENTS: a fundamental principle in the control of the disease is phytosanitary roguing, based primarily on the fact that the diseased palm is the major source of inoculum and the niche for vector development. After confirmation that a tree has red ring disease (see below), the leaf axils should be sprayed with 0.1% Lannate (methomyl) solution to kill off the palm weevils living in the crown (Griffith, 1971). Trees should be killed with 100–150 ml (48.3% a.i.) of the herbicide monosodium acid methanearsonate (MSMA) or other herbicide that is injected or placed into the trunk. This usually takes 2–3 weeks. Occasionally, trees injected with MSMA will harbour weevil larvae. Thus, the tree should be cut and sectioned to make sure that weevils are not present. When trees are discovered in advanced stages of the disease, or when they are seen in a 'broken neck' condition, they cannot be poisoned with herbicides. Such trees should be cut down and the pieces and remaining stump sprayed thoroughly with an insecticide, such as methomyl, trichlorfon, monocrotophos, carbofuran, carbaryl, imidacloprid or lindane. If the tree is sprayed adequately with an insecticide, all larvae and pupae of the palm weevil that were developing in the diseased tree need to be killed. It is recommended that the site be checked every couple of weeks

until the palm has decomposed, or that the dried-out remains of the palm be burned with the aid of kerosene.

MASS TRAPPING OF PALM WEEVILS: phytosanitation (the removal and destruction of red ring diseased trees) and trapping of weevils using pesticide-treated palm or fruit tissue have been the recommended methods of management of red ring disease in coconut for many years (Mariau, 1968; Griffith, 1969; Delgado and Moreno, 1986). Trapping becomes significant in reducing the abundance of palm weevils generally and, dependent on the density of traps per hectare, catch the smaller percentage of vector weevils that generally visit and infect palms in the near vicinity, one or two trees away, from the source of infection. The identification, synthesis and commercial availability of male-produced aggregation pheromones of palm weevils have improved significantly the efficacy of the older methods of trapping with fermenting tissue alone (Oehlschlager *et al.*, 1993, 2002; Moura *et al.*, 2000). Although more research is needed in coconut, Moura *et al.* (2000) demonstrated in Brazil that by using 54 100-l perimeter traps of pheromone (Rhyncholure; racemic 6-methyl-2-hepten-4-ol; ChemTica International, Costa Rica)³ plus sugarcane around a red ring diseased coconut plantation (54 ha) for 26 months, the *R. palmarum* capture rate (>97,000 weevils were captured during this period) remained constant, but the red ring disease incidence dropped dramatically. In Tabasco, Mexico, using pheromones in guerrero-type coconut tissue traps over several months showed a positive correlation with the number of insects captured and the incidence of disease (Perez-Marquez, 1999, personal communication). Future research in the management of red ring disease in coconut palm, which is a very suitable and susceptible host for red ring disease and *R. palmarum*, must examine the efficacy and cost-effectiveness of perimeter mass trapping in concert with phytosanitation in different situations (small versus large crop holdings of various aged palms) in rural tropical America.

BIOLOGICAL CONTROL MEASURES: natural enemies have not been evaluated thoroughly for the management of potential palm and sugarcane weevil vectors. Several organisms may hold

promise, including entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae, the prokaryote *M. roseus*, tachinid parasites, *Billaea rhynchophorae* and *Billaea menezesi*, and the predatory histerid beetle, *Oxytetrus maximus* (references cited in Giblin-Davis, 2001; Löhr, 2012).

Methods of diagnosis

RECOVERY OF *B. COCOPHILUS* FROM COCONUT TISSUE: the well-established methods for obtaining samples of nematodes from living trees are still used. A stainless steel tube, sharpened at one end, is driven at an angle of 45° at the point selected for sampling. The extracted core is placed in a blender with 50 ml of water and processed for 2 min. The contents of the blender are then poured into a dish and left for 20 min for the nematodes to emerge. The nematodes are then recovered by sieving. The red ring nematodes are often highly mobile in water (swimming and coiling), leading to knots of clumped nematodes or resuspension of the nematodes after centrifugation. In coconut and the palm-iste palms, the nematodes are most active in the stem tissue, except in the very necrotic regions. The core tissue generally shows a red cylinder of necrotic red ring tissue. In the method originally used by Fenwick and Maharaj (1963), diseased coconut stem, petiole or root tissue is chopped into fine pieces about 1 cm in thickness, placed in a large funnel of water, whose stem is closed at one end with a tube and clip, and whose neck has a small plug of cotton acting as a filter. This can be modified by macerating the diseased tissue in a blender to release more nematodes and then screening through a No 400 USA Standard Testing Sieve (38 µm openings) before backwashing into the funnel. The funnel is allowed to stand overnight before harvest. Schuiling and Van Dinther (1981) offer another modification for extracting red ring nematodes from tissue.

Radopholus similis

The burrowing nematode *R. similis* occurs in most tropical and subtropical areas of the world and has been reported from coconut palms in Florida, Jamaica, Sri Lanka and India (see Griffith

et al., 2005, for references). Koshy (1986) suggested co-evolution of the nematodes along with black pepper and certain cultivars of banana in the western hills of south India. It occurs deep inside the forests on wild black pepper and is widespread on a number of crops such as coconut, arecanut, black pepper, banana, betel vine and ginger in south India.

Symptoms of damage

The burrowing nematode causes non-specific general decline symptoms such as stunting, yellowing, reduction in number and size of leaves and leaflets, delay in flowering, button shedding and reduced yield. *R. similis* infestation produces small, elongate, orange-coloured lesions on tender creamy-white roots. Consequent to nematode parasitization and multiplication, these lesions enlarge and coalesce to cause extensive rotting of the roots (Fig. 14.5). Tender roots of coconut seedlings with heavy infestation



Fig. 14.5. Roots of arecanut palm showing lesions, blackening and rotting due to *Radopholus similis*. (Photograph courtesy of V.K. Sosamma.)

become spongy in texture. Surface cracks develop on the semi-hard, orange-coloured main roots. Lesions and rotting are confined to the tender portions of the root. Lesions are also not conspicuous on the secondary and tertiary roots, since these are narrow and rot quickly on infestation.

As many as 4000 nematodes can occur in 1 g (2.5 cm length) of main roots. The nematode also attacks the plumule, leaf bases and haustoria of seedlings. The above-ground symptoms being non-specific, the only definite method to identify an infested palm is to look for characteristic lesions on fresh, creamy-white to orange-coloured tender main roots after cleaning and rubbing the epidermis.

R. similis does not enter or penetrate the coconut roots that have developed a hardened or suberized epidermis, but does penetrate and lyse cells in the absorbing region behind the root cap covered by a very delicate epidermis. The cavities that form in the outer cortex are always surrounded by deeply stained and heavily suberized cells of irregular shape, whereas those formed in the inner cortex do not have any such deformed, darkly stained border cells. The maximum numbers of nematodes and cavities are seen in the outer cortex. Nematodes have not been observed in the stelar region or in the closely packed four to six layers of cells outside the endodermis, even in heavily infested roots. In the early stage of infection, roots have separate cavities that later merge with each other consequent to the feeding and multiplication of nematodes.

Multiple cavities and their coalescence destroy the cortex to a great extent, but the stelar tube remains intact. Eggs and all stages of nematodes with different orientations are seen in the cavities in longitudinal sections (Koshy and Sosamma, 1982a, 1987; Sosamma and Koshy, 1991, 1998).

Biology and life cycle

The burrowing nematode is a migratory endoparasite and is capable of spending its entire life within roots. Most juvenile stages and adult females, including gravid females, infest healthy, succulent root tips; fourth-stage and adult males do not. The nematode takes 25 days at 25–28°C to complete one life cycle (J2–J2) (Geetha, 1991).

The coconut isolate of *R. similis* from Kerala, India, is the 'banana race', as it does not infest

Citrus spp. or *Poncirus trifoliata* (Koshy and Sosamma, 1977) and has a haploid number ($n = 4$) of chromosomes (Koshy, 1986; Jasy, 1991). The *R. similis* population from coconut root is easily cultured axenically on carrot discs placed on 1% water agar or 10% tapioca pearl (Koshy and Sosamma, 1980; Banu and Sosamma, 1999). It can also be cultured within the mesocarp of growing tender coconuts without affecting the size or quality of the nuts (Koshy and Sosamma, 1982b).

Survival and means of dissemination

The burrowing nematode survives under field conditions for 6 months in moist soil (27–36°C) and for 1 month in dry soil (29–39°C); under glasshouse conditions, it survives for 15 months in moist soil (26–29°C) and for 3 months in dry soil (27–31°C). The nematode survives in the roots of stumps of felled coconut palms for up to 6 months (Sosamma and Koshy, 1986), and as adult females in coconut roots and soil during summer months, causing annual recurrence of infection (Sosamma, 1984).

Coconut seedlings are raised by sowing seed nuts in the interspaces in coconut plantations in Kerala, India. Most of the nurseries in Kerala and Tamil Nadu (south India) are infested by *R. similis* (see references in Griffith *et al.*, 2005). One-year-old coconut saplings raised in these infested nurseries harbour large populations of the nematode in roots internal and external to the husk. Such seedlings when distributed for planting help in the dissemination of the nematode over long distances (Koshy and Sosamma, 1978b, 1979).

Environmental factors affecting parasitism

Infested coconut roots yield a maximum number of *R. similis* during October to November and a minimum number during March to July in India. Factors favourable to nematode multiplication are a mean soil temperature below 25°C and a light rainfall, coupled with the availability of tender, fleshy roots. Nematode populations in the roots of individual palms were found to vary considerably during low and high peaks, depending on the age, cultivar and conditions of the palms involved (Koshy and Sosamma, 1978a). The burrowing nematode multiplies

well on coconut in loamy sand, followed by riverine alluvium, but least in Kari-type soils. However, it causes maximum plant damage in riverine alluvium and the lowest in laterite soil (Sosamma, 1984; Sosamma and Koshy, 1985).

Other hosts

The coconut isolate of *R. similis* has a wide host range including several economically important plants, weeds and trees. Of 115 plant species tested, 48 species belonging to 45 genera in 17 families were recorded as hosts (Koshy and Sosamma, 1975; Sosamma and Koshy, 1977, 1981).

Disease complexes

The fungi *Cylindrocarpon effusum*, *Thelonectria lucida* and *Cylindrocladium clavatum* have been recorded in association with lesions produced by *R. similis* in coconut roots. In pathogenicity studies, the fungus *C. effusum* did not cause any appreciable damage to inoculated seedlings. The fungus, when inoculated simultaneously with the nematode, reduced the rate of multiplication of the nematode and damage to coconut seedlings (Sosamma and Koshy, 1978, 1983; Koshy and Sosamma, 1987; Sosamma, 2000b). *Aphelenchoides aligarhiensis*, *Panagrolaimus rigidus* and *Rhabditis* sp. were isolated from leaf rot disease-affected spindle leaves of coconut in Kerala, India (Nadakkal, 1965; Sosamma, 2000c). The application of phorate at 2 g a.i./palm to the base of the unopened spear leaf was helpful in controlling the disease. However, the role of nematodes in the disease complex as passive vectors/synergists has not been defined (Koshy, 2000; Koshy *et al.*, 2002c).

Economic importance and population damage threshold levels

Surveys of different coconut-growing tracts of Kerala, Karnataka and Tamil Nadu states of India (964,000 ha) revealed the widespread occurrence of *R. similis*. Of the root samples taken, 24% yielded *R. similis*, and of these, 50% yielded one or more *R. similis*/g of root (Koshy *et al.*, 1978; Sosamma, 1984). A 30% increase in yield was recorded by the application of *Hydrocarpus* sp. oil cake at 8 kg/palm/

year or phorate and aldicarb at 10 g a.i./palm in June–July and October–November to the burrowing nematode-infested coconut palms (Koshy, 1986).

The pathogenicity of *R. similis* on coconut was established by conducting two experiments, the first with a duration of 5 years and the second over a period of 1 year (Koshy and Sosamma, 1987). An initial inoculum level of 62,500 nematodes/seedling caused 4, 22, 76, 18, 25, 40, 48 and 79% reduction with respect to height, girth at collar region, shoot weight, number of leaves, number of leaflets/leaf, leaflet length, lamina length and root weight, respectively, over the control plants. The effect of parasitization of the nematode was more pronounced on the root system, especially on the number and mass of feeder roots. The threshold inoculum density required for causing significant reduction of various growth parameters was 100 nematodes in 625 cm³ or 900 g of soil under field conditions over a period of 5 years.

In the second experiment, an initial inoculum level of 100,000 nematodes caused 40, 55, 20, 65, 20, 48 and 52% reduction with respect to height, shoot weight, number of leaves, leaf area, number of lateral roots, volume and weight of roots, respectively, over control plants over a period of 1 year. Leaf bases and haustoria of seedlings were also infested by nematodes. No appreciable damage was noticed in plants inoculated with the fungus *C. effusum* alone. The pathogenic threshold level of the axenic *R. similis* population for causing damage to all plant growth parameters was 1000 nematodes/seedling or 10 nematodes/100 cm³ or 140 g of sandy loam soil under greenhouse conditions. The histopathology of infested roots recorded the presence of nematodes in the cortex in the inter- and intracellular positions (Koshy and Sosamma, 1983, 1987; Sosamma, 1984).

To facilitate normal growth of the plant to flower and exhibit the disease under natural conditions, a detailed pathogenicity trial was initiated in 1.8 m × 1.8 m × 1.2 m field tanks (microplots) over a period of 11 years using axenic inoculum. This experiment characterized the damage potential of burrowing nematodes on coconut. All the uninoculated palms came to flowering during 65–83 months after planting, between leaf axils 31 and 49, whereas four out

of the five palms that received an initial inoculum level of 100 nematodes flowered during 67–130 months in the leaf axils from 39 to 56. Two palms each that received an initial inoculum level of 1000 and 10,000 nematodes came to flowering after 108 months, and one out of five palms that received an initial inoculum level of 100,000 nematodes came to flowering after 132 months. None of the palms that received 1,000,000 nematodes came to flowering. The control palms produced a total of 155 inflorescences compared with 67 inflorescences in palms inoculated with 100 nematodes as an initial inoculum level. However, the palms that received an initial inoculum of 1000 nematodes and above did not yield any nuts, even after 11 years. The control plants produced an average of 125 nuts compared with 37 nuts by palms that were inoculated initially with 100 nematodes. Even one nematode in 35,640 cm³/soil or 100 nematodes/seedling reduced the yield by 77% (Koshy and Sosamma, 1994, 1996).

Management measures

Management of the burrowing nematode on a perennial palm such as coconut with a massive root system is difficult, especially under the high-density, multispecies cropping system that exists along the west coast of south India involving susceptible crops such as arecanut, banana, black pepper, betel vine, ginger and turmeric. Intensive use of nematicides for the control of the burrowing nematode may cause problems of residual toxicity in coconut water and copra (Habeebullah *et al.*, 1983; Sosamma, 1996). Apart from this, it may also lead to residual toxicity in the products of the intercrops. Therefore, control of nematodes by field application of nematicides alone is not a practical proposition.

CULTURAL PRACTICES: the cultural practices existing in Kerala and Karnataka (India) are the application of neem and marotti (*Hydrocarpus*) oil cakes at 2–4 kg/palm/year, farmyard manure at 50 kg/palm/year, and green foliage and tender stem of the tropical forage tree *Gliricidia maculata* to the basins at 50 kg/palm/year. Growing green manure crops such as cowpea, *Crotalaria* or *Sesbania* in the basins and interfaces during June to August and ploughing in the

entire crop at flowering help in reducing the burrowing nematode population and enriching the nutritional status of the soil. In addition, growing intercrops such as cacao that enrich the soil with sizeable quantities of shed foliage helps in the build-up of beneficial organisms and antagonistic microorganisms that may inhibit nematode multiplication (Koshy *et al.*, 1991a,b, 2002a).

BIOLOGICAL: a significant increase in width and leaf area has been recorded in coconut seedlings that received mycorrhizae alone. An increase in shoot weight, root weight and a decrease in lesion indices occur in seedlings inoculated with mycorrhizae prior to *R. similis*. A mixture of mycorrhizae consisting of multiple endophytes, i.e. *Acaulospora bireticulata*, *Glomus fasciculatum*, *Glomus macrocarpum*, *Glomus mosseae*, *Glomus versiforme*, *Sclerocystis rubiformis* and *Scutellospora nigra*, was found effective in improving the plant growth and reducing *R. similis* infestation of coconut seedlings (Sosamma, 1994).

Minimum growth characters and maximum multiplication of nematodes were recorded in plants that were inoculated with *R. similis* alone. In combined inoculation of mycorrhizae and nematode, maximum growth was recorded in plants inoculated with *A. bireticulata*. *A. bireticulata* had maximum multiplication on coconut compared with *G. macrocarpum*, *Scutellospora coralloidea* and *S. rubiformis* in nematode-free as well as nematode-inoculated plants. Nematode populations were also lower in plants inoculated with *A. bireticulata* (Sosamma, 1994; Sosamma *et al.*, 1998a).

A new isolate of *Pasteuria* parasitizing *R. similis* in Kerala, India, has great potential for use in integrated pest management. The infective propagules of *Pasteuria* adhered to the cuticle of males, females and juveniles of *R. similis* (Sosamma, 1999, 2000b,d, 2002). The introduction of *Purpureocillium lilacinum*, *Pasteuria penetrans* and mycorrhizae into potting mixture contained in plastic bags in coconut nurseries and again in the planting pit at the time of transplanting coconut seedlings in the field improved the establishment of plants and imparted better growth by protecting against *R. similis* (Koshy, 1998; Koshy *et al.*, 1998a; Sosamma *et al.*, 1998b). *Catenaria vermicola* was also found parasitizing *R. similis* in Kerala (Sosamma, 2000a).

RESISTANCE AND TOLERANCE: all the coconut cultivars (29 exotic, 15 indigenous and 15 hybrids) screened for resistance to *R. similis* in India were found susceptible to varying degrees. The dwarf cultivars Kenthali and Klappawangi recorded the least nematode multiplication and lesion indices. Similar reactions were noticed in hybrids such as Java Giant $\times$ Kulasekharam Dwarf Yellow, Java Tall $\times$ Malayan Yellow Dwarf and San Ramon $\times$ Gangabondam (Sosamma *et al.*, 1980, 1988; Sosamma, 1984).

CHEMICAL: burrowing nematode infestation in coconut nurseries has been detected in India. Increased incidence of *R. similis* can occur when banana is used as a shade crop in coconut nurseries. In these situations, there is possibly a case for the treatment of nurseries with nematicides to produce nematode-free seedlings to prevent spread of the nematode into the main field and to uninfested areas.

Past experience has shown that a dip in 1000 ppm dibromochloropropane (DBCP) for 15 min is effective in controlling nematodes in seedlings for *R. similis*-infested coconut nurseries (Koshy and Sosamma, 1978b, 1979). Complete control of *R. similis* can be obtained with soil application of phenamiphos or phorate at 25 kg a.i./ha during May, September and December in infested coconut nurseries (Koshy and Nair, 1979; Koshy *et al.*, 1985).

Summary of management measures

The following measures are suggested towards developing an integrated management schedule for *R. similis* infestation on coconut palms (Koshy, 2002):

- Application of cow dung (50 kg), oil cakes (2–4 kg) and green manuring with *G. maculata* (50 kg) per palm/year to the basins.
- Growing *Crotalaria juncea*, cowpea or *Sesbania* in the basins and interspaces and incorporating into the soil by ploughing in at flowering stage.
- Application of phorate at 10 g a.i./palm twice yearly (in June–July and in October–November in India).
- Avoid growing bananas as a shade crop in coconut nurseries.
- Use of nematode-free planting material of coconut and other intercrops.
- Use of tolerant or less susceptible cultivars or their hybrids in infested areas.
- Cut and remove all roots external to the husk of seedlings raised in the field before planting.
- Raise coconut seedlings in potting mixture enriched with bioagents such as *P. lilacinum*, *P. penetrans* and mycorrhizae in plastic bags.
- Introduce bioagents into the planting pits while planting in the main field.
- Apply phorate at 3 g a.i./plant to intercrops such as banana, black pepper and arecanut in June–July and October–November.

Methods of diagnosis

SAMPLING: soil and root samples for the detection of *R. similis* should be collected when maximum populations of the nematode occur (October–November in India). Maximum populations of *R. similis* are found on coconut at a distance of 100 cm from the bole of the palm and at a depth of 50–100 cm. Fifty g of tender, creamy-white to orange-coloured, semi-hard main roots (~1 cm diameter) showing lesions and rotting should be collected to obtain live populations in large numbers (Koshy *et al.*, 1975).

EXTRACTION: the semi-hard, orange-coloured main root bits are peeled and sliced longitudinally into 4–8 pieces of 3–5 cm length. These sliced root bits are submerged in water contained in Petri dishes or shallow pans; a temperature of 20–25°C is ideal for the extraction of live nematodes from polyphenol-rich coconut roots (Koshy *et al.*, 1975). After every 24 h of incubation, the water needs to be changed; 50% of the population is extracted after 72 h. Most of the nematodes are recovered within 4–7 days.

DETERMINATION OF POPULATIONS AND CROP LOSS: nematode populations in the tender portions of the main roots can be estimated by staining and blending. Roots may be cut into 2-cm long pieces, sliced longitudinally into eight sections and then stained.

Nematodes for the Control of other Coconut Pests

Entomopathogenic nematodes *Heterorhabditis indica* and *Steinernema* spp. were isolated from soil around coconut in Kerala and were used in the integrated management schedule for the Rhinoceros beetle, *Oryctes rhinoceros*, and the red palm weevil, *Rhynchophorus ferrugineus* (Sosamma and Banu, 1996; Banu *et al.*, 1998; Sosamma, 2000b, 2003).

Conclusion and future prospects

The burrowing nematode *R. similis* is second in importance to the red ring nematode *B. cocophilus* on the basis of its damage potential on coconut. Although the nematode has been reported in association with various coconut diseases, no detailed investigations have been carried out anywhere else except in India. Screening for resistance/tolerance to *R. similis* in coconut cultivars and their hybrids has indicated the availability of possible resistance in some cultivars. Although breeding in coconut is a long-term process, this area could be exploited profitably. Developing an integrated management schedule for the coconut based on subsistence farming systems involving susceptible perennial crops such as arecanut, black pepper, cacao and banana should be a priority area of research.

Oil Palm

The oil palm *E. guineensis* has a natural distribution in West Africa between latitudes 13°N and 12°S from the coast to the Great Lakes. Ecologically, it is found in the transition regions between the rainforest and the savannah. It has also been cultivated extensively in Malaysia and Indonesia. Commercial production of oil palm in Central and South America dates back only to the 1960s, although production is expanding in all of tropical South America. In the New World, it is a plantation crop with holdings of several hundred to several thousand hectares per unit, whereas in Africa or Asia it can be a large plantation or a smallholder's crop, as with coconut.

Nematodes of Oil Palm

Generally, the major diseases of the oil palm are found in its area of origin. Curiously, although there are fungal-, bacterial- and suspected viral- or phytoplasma-induced diseases, no records of any economic losses due to nematode damage occur in the Old World. However, *B. cocophilus* causes economic loss in oil palm in Central and South America. Other plant parasitic nematodes have been reported on oil palm in India, Pakistan and other countries (see references in Griffith *et al.*, 2005).

Bursaphelenchus cocophilus

In Trinidad in 1925, Freeman appears to have been the first to record the pathogenicity of the red ring nematode on oil palms. Red ring disease caused by *B. cocophilus* has been known from African oil palm from Venezuela since before 1953 (Webster and Gonzalez, 1959) from a single plantation of 1000 ha, where the disease caused severe losses. Malaguti (1953) demonstrated that African oil palm, which at that time had recently arrived in Latin America from Africa, was invaded by the red ring nematode. Malaguti cites a group of 100 palms showing only 16 doubtful cases in January, but which by August had 22 deaths, 9 doubtful or affected cases and only 69 palms remaining healthy. On that Venezuelan estate, about one-third of the total palm population became infected.

Symptoms of red ring disease in oil palms and biology

The coloration in the diseased palm is similar to that of the browning associated with the 'nana' or dwarf cultivar of coconuts, i.e. brownish rather than reddened tissue internally. Also, the leaves dry out and turn brown instead of the usual yellowing and then browning associated with the tall cultivar of coconuts (Figs 14.6 and 14.7). Often, the centre of the crown takes on a dwarfed appearance, and the newly opened 'little leaves' become bundled together, the leaflets being twisted, corrugated and adhering to a stiff upright rachis (Figs 14.6 and 14.7). The developing bunches show necrosis, and the



Fig. 14.6. (a) Premature death, browning and retention of older leaves of oil palm associated with red ring disease caused by *Bursaphelenchus cocophilus*. (Photograph courtesy of K. Gerber.) (b) Fern-covered oil palm with little leaf caused by *Bursaphelenchus cocophilus*. (Photograph courtesy of R. Giblin-Davis.)

inflorescences do not set fruit. The ultimate symptoms of red ring disease in oil palm are similar to those of the coconut palm, but there are some fundamental differences in the progress of the infection that can lead to new and distinct measures for treating the disease in the crop.

Pathogenesis is longer than in the coconut, generally 5–10 months in the more susceptible cultivars. In the coconut, the young 3- to 10-year-old palm is virtually dead within 3 months of infection. In the case of the oil palm, this process can take up to 3–4 years with a palm of the same age group. This is partially because the nematode does not colonize as rapidly in the oil palm tissue as it does in the coconut. Where 5000–10,000 nematodes/g of tissue can be found in the red ring zone of coconut, a similar region in the oil palm often yields less than 500 nematodes/g of tissue. A further difference is that most nematodes are found outside the necrotic zone, even in areas that show no necrosis, such as the distal or basal portion of the stem and

occasionally in the rachis of the inflorescence (the nematode has not been isolated from the rachis of the coconut panicle). In addition, the red ring disease infection rate is often highest in older oil palms (15 to >20 years old) (Oehlschlager *et al.*, 2002).

As in the case of the coconut, the most persistent form of the nematode is the third-stage juvenile, which can subsist for a long time in the diseased tissue. In the coconut, this juvenile form readily proceeds to the adult in the healthy tissue not showing symptoms. However, in the oil palm, this interval is prolonged for some reason, with the result that colonization of the oil palm is not rapid and pathogenesis is attenuated. A notable feature in accordance with this is that the band of necrotic tissue is usually very narrow and often irregular in shape (Chinchilla, 1988). Eggs appear as usual in the brownish spots that are present in the advancing area of the disease. Such necrotic areas indicate evidence of plant reaction to the cellular damage

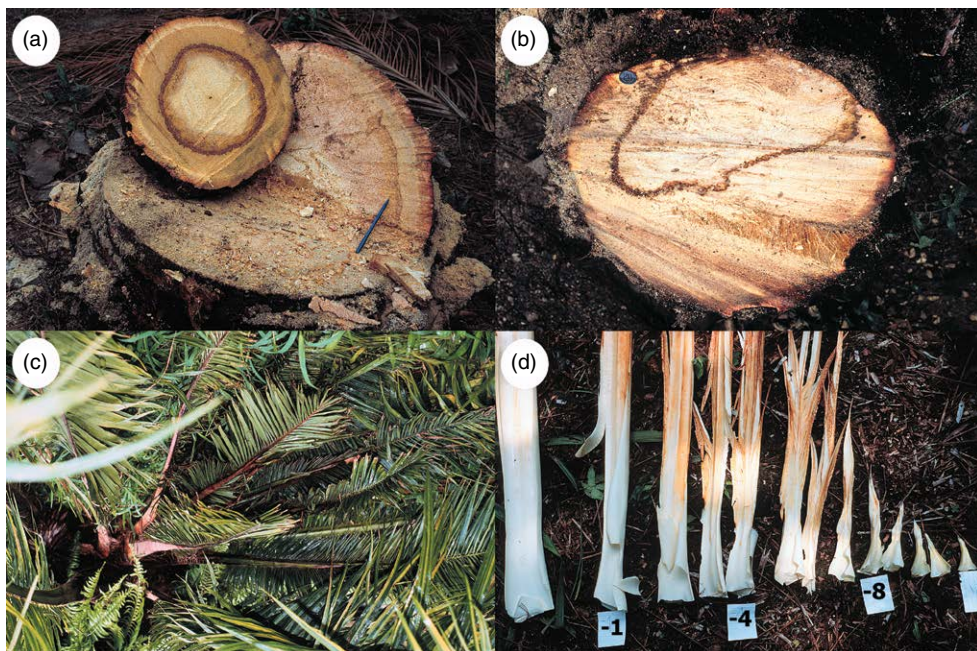


Fig. 14.7. (a) Brownish ring of diseased tissue caused by *Bursaphelenchus cocophilus* in cross sections of oil palm stem at different heights. (Photograph courtesy of K. Gerber.) (b) Irregular brownish ring caused by *Bursaphelenchus cocophilus* in cross section of oil palm stem. (Photograph courtesy of R. Giblin-Davis.) (c) Little leaf symptoms in crown of oil palm caused by *Bursaphelenchus cocophilus*. (d) Dissected crown of little leaf symptomatic oil palm with spear leaf and the unopened leaves counting backwards with necrotic lesions caused by ectoparasitic feeding by *Bursaphelenchus cocophilus*. (Photographs courtesy of R. Giblin-Davis.)

caused by the abundance of the nematode. The nematodes often show no evidence of their presence, and an abundance of nematodes can occur without the plant reacting visibly. Yet, artificial inoculation studies have demonstrated that increased logarithmic strengths of the inoculum correlate inversely with the length of the period for pathogenesis in 8-year-old palms at Centeno, Trinidad.

The palm weevil, *R. palmarum*, as with coconut, is the main vector of *B. cocophilus*. The canopy in an oil palm plantation is always closed, with reduced light intensity and more humidity than in coconuts. This presents ideal conditions for the vector of the nematodes, the palm weevil, which is crepuscular.

In the state of Amazonas, Brazil, the number of weevils infested with nematodes showed high monthly variations and irregular distribution, with higher percentages occurring in November 1988 and September 1991. The relatively low

incidence of red ring disease in the area did not suggest any association between the disease and the variations in rainfall measured (Araujo *et al.*, 1998).

Spacial disposition of diseased oil palms

In young 5- to 10-year-old groves, there is a tendency for the diseased palms to be clustered in a 50 m radius that gradually expands. In older groves, however, the diseased trees appear to be distributed at random, giving the impression that the vectors come in from fields that are more susceptible to the simultaneous development of both the nematode and the palm weevil. The major constraint is the poor opportunity for association of the developing weevil larvae in the oil palm with a large number of nematodes. This is a result of the slower rate of colonization of the nematode in the oil palm compared with the coconut palm. Therefore, unlike a coconut estate, where most

weevils develop in diseased trees that become the main or focal developmental niche (97.3% in Trinidad), the proportion, in oil palm fields, would relate more to the Ecuador situation, with greater percentages of the weevils developing outside the ambit of the nematode. This is markedly so as the incubation period of the pathogen in oil palm is very long and quite variable. The principle of the diseased palm being the main attractive focus for palm weevils would have reduced relevance here; thus, weevil lures would assume a more significant role in disease management in the field. Consequently, weevil trapping in oil palm estates is an important form of control for reduction of the disease. Moreover, the location and elimination of sources of infection, other than oil palm, particularly diseased coconut fields, near the affected grove is important. Phytosanitary measures, however, comprise the most utilized method of control in Latin America.

Other hosts for red ring disease in some oil palm estates in Brazil

The wild palm *Oenocarpus distichus* was found by Schuiling and Van Dinther (1981) to be capable of contracting red ring disease and serving as a host for *R. palmarum*. This is a typical palm of primary and secondary forest of the Amazon estuary. Nematodes are often fewer in number, often less than 100/g of tissue. However, palm weevil larvae found growing in these trees were contaminated internally with red ring nematodes. The weevil *R. palmarum* is reported from 31 plant species, belonging to 12 families, with palms being the main hosts (Sanchez and Cerda, 1993).

Economic importance and damage threshold levels

In Latin America, there is an apparent direct correlation between the levels of red ring disease in coconuts and those in oil palms. Countries with high levels of red ring disease in coconut groves also have high levels of red ring in oil palm groves. Generally, in oil palms 8–10 years old, the incidence is around 0.1%, and in palms over 20 years old, the incidence is rare. However, in some zones adjoining old coconut establishments, the incidence of disease in oil palms 11–18 years old can be as high as 30%. In one parcel of 62 ha of the plantation of Palmeras de la Costa in Colombia, the maximum accumulated disease total for 1987 was 8.3% (Griffith *et al.*, 2005).

Little leaf disease of oil palms

The oil palm, as most palms, has a tendency towards producing so-called 'little leaves', the cause of which may be diverse and related to symptoms of other diseases. In Surinam, Van Hoof and Seinhorst (1962) observed that little leaf syndrome was associated with attack by the red ring nematode. Little leaf symptomatic trees can be recognized easily by their erect, short and often deformed leaves with suberized patches, especially on the inner side of the leaf stalks (Chinchilla, 1988).

Many *B. cocophilus* have been found on discoloured tissue of young (up to 1.75 m long), folded leaves, still protected from the sun. The nematodes live ectoparasitically in the buds of the palms. In one survey of 50 diseased oil palms cut for investigation, only one did not contain nematodes. *B. cocophilus* was never found on the young leaves of numerous trees that did not suffer from little leaf but were cut for other reasons (Van Hoof and Seinhorst, 1962). Palms exhibiting this symptom can live for many years, but with a reduced leaf emission rate and abortion of inflorescences (Chinchilla, 1988). It has been hypothesized that red ring nematode-induced little leaf is symptomatic of unsuccessful cases of the systemic red ring disease and is more common in African oil palm than in coconut, where it is rarely observed because coconut is so susceptible and succumbs so easily to red ring disease (Giblin-Davis, 1993).

Management of red ring in oil palm

Generally, the control of red ring disease in oil palm is similar to that in coconut, by a combination of methods. The destruction of diseased trees is paramount as soon as the symptoms are detectable, in order to destroy inoculum.

Injections of systemic nematicides, such as fenamiphos, oxamyl and carbofuran, into little leaf symptomatic palms can help with palm recovery. However, because of the damage to the very young leaves in little leaf palms, recovery can take between 6 and 8 months (Chinchilla, 1988). This measure is unsuccessful in coconuts because the nematode colonizes the palm tissue too rapidly, but in oil palm, the slower rate of colonization allows for such a possibility to control the nematode directly. Non-target effects and cost-effectiveness must also be considered, and little leaf symptomatic oil palms are usually better removed, providing

more light into the canopy and increased productivity to nearby palms (Chinchilla, 1988).

Research by Oehlschlager *et al.* (1992a, 1993, 1995, 2002) in African oil palm plantations in Costa Rica suggests that concerted aggressive phytosanitation and mass trapping with traps baited with sugarcane and synthetic aggregation pheromone (Rhyncholure; racemic 6-methyl-2-hepten-4-ol; ChemTica International) reduce the numbers of *R. palmarum* and change their distribution patterns (from highly aggregated to random), while significantly reducing red ring disease incidence. Initial bimonthly inspections and the removal of red ring diseased palms did not reduce red ring disease incidence in two large African oil palm plantations in Costa Rica. One year after the initiation of mass trapping at trap densities of about one trap per 5 ha, red ring disease incidence plummeted by more than 80% (Oehlschlager *et al.*, 2002). At mass trapping onset, most *R. palmarum* were captured in 'border' traps on test sites, suggesting the removal of potential immigrants into the study area. A combination of perimeter and 'internal' traps appears to be most effective for mass trapping in African oil palm (Oehlschlager *et al.*, 1995). There are many trap designs available for effectively capturing palm and sugarcane weevils (Oehlschlager *et al.*, 1993; Giblin-Davis, 2001). The most important features involve baiting with sugarcane or palm tissue (changed every 2 weeks) and a pheromone release device, and making the trap lethal with pesticide treatment of tissue or by using a special trap design with soapy water. The traps must be examined and refreshed at least every 2 weeks with fresh pesticide-treated sugarcane tissue and pheromone (as needed). The addition of ethyl acetate ('weevil magnet')² can improve pheromone trap capture efficiency (Giblin-Davis *et al.*, 1996).

Studies to determine whether *Metamasius* spp. is a vector of *B. cocophilus* did not achieve transmission of red ring in oil palms, but the frequency of the nematodes occurring in the insects was significant (Silva, 1991).

Methods of diagnosis

The methods for extraction of *B. cocophilus* from oil palm are similar to those described for the nematode in coconut; however, they are potentially less

accurate because of the lower numbers of nematodes present and the often irregular shape of the rings in the oil palm (Chinchilla, 1988). Nematodes also seem to thrive in the petioles.

Date Palm

The date palm *P. dactylifera* is dioecious, and artificial pollination by humans has played a significant role in the historical development of the crop. Tissue culture programmes have become important for improving yields. In 2011, the FAO estimate of worldwide production of dates was 6,893,280 Mt. The main contributors were: Egypt, which produced 1,373,570 Mt; Saudi Arabia, 1,122,820 Mt; Iran, 1,016,610 Mt; United Arab Emirates, 900,000 Mt; Algeria, 690,000 Mt; Iraq, 619,000 Mt; and Pakistan, 557,280 Mt. Although the palms will grow throughout the tropics, the number of heat units required from the time of blossoming to ripening needs to be between 4000 and 5500 for various cultivars. Growth of the palm ceases around 10°C. Suitable climatic conditions occur in the southern deserts of California, USA, where the palm has been grown successfully on a commercial scale in the Coachella Valley. In this introduced environment, the palm has to cope with the new prevailing nematode fauna.

Nematodes of Date Palm

The date palm is affected by numerous pests and diseases wherever it is grown, but nematodes parasitic on date palm, with the exception of root knot nematodes, *Meloidogyne* spp., have not been well studied. However, nematodes have not generally been found to be a limiting feature in the countries with date as an ancient culture. Root knot nematodes, *Meloidogyne* spp., were found in the Coachella Valley of California on date palms in 1925, where they are now known to be widely distributed in commercial date plantings. Buhner *et al.* (1933) first reported the occurrence of root knot nematodes on date, and Jensen (1961) found *Meloidogyne incognita* on roots of date palms in nurseries. Carpenter (1964) reported that root knot nematodes, principally *Meloidogyne javanica*, could severely damage or kill date palm seedlings.

Young seedlings of 50 date cultivars were susceptible to infection by root knot nematodes; more than 90% of the seedlings were killed prior to emergence when seeds were sown in heavily infested soil. Secondary damage by fungi to roots of field-grown palms infested with the nematodes seemed to be an important factor in the deterioration and death of roots. Minz (1958) reported the occurrence of *Meloidogyne arenaria*, *Meloidogyne hapla*, *M. incognita* and *M. javanica* on date palms in Israel. *Meloidogyne* spp. have been reported from Sidi Yaia in Algeria (Lamberti *et al.*, 1975), from the Mauritanian oases of Tayaret and Tejitt (Netscher and Luc, 1974), Oman (Mani *et al.*, 2005), Egypt (Youssef and Lashein, 2013; Youssef, 2014) and from Libya (Fourgani and Edongali, 1989; Edongali, 1996).

The combination of *Ceratocystis paradoxa* with *M. javanica* increased the susceptibility of date palm cultivars to infection by the fungus (Aboud *et al.*, 2002).

Histopathological studies of date palm (*P. dactylifera*) roots infected with *Pratylenchus penetrans*, the root lesion nematode, showed the puncture of epidermal cells and disarrangement of cortical cells with large, empty, abnormal cavities. Membranous cell walls were wavy and collapsed as the supporting material was destroyed by nematode infection (Khan *et al.*, 2002).

The cellular alteration in *M. incognita*-infected roots of susceptible and resistant date palm cultivars was histologically studied in pot experiments. Giant cell formation was favoured in the susceptible cv. Zaghloul, while in resistant cvs. Deglet Noor and Samani, the infected roots reacted to the nematode infection by forming a necrotic area around the invading nematode. In certain cases, malformed small giant cells were observed in association with nematode juvenile stages (Eissa *et al.*, 1998). In Egypt, the largest *M. incognita* populations were found at soil depths of 30–50 and 51–70 cm at 1 and 2 m distances from the trunk of date palm cv. Siwi (Youssef and Eissa, 1994). Thirty-seven species of plant parasitic and free-living nematodes were encountered on date palm in India. Date palm trees infected with *M. incognita* (450 second-stage juveniles/250 cm³ of soil) showed yellowing of leaves and stunted growth (Lal and Mathur, 1986).

A survey of plant parasitic nematodes in the rhizosphere of 30 date palm cultivars in Riyadh, Saudi Arabia, found 18 genera of plant parasitic

nematodes in the following descending order of frequency: *Helicotylenchus* (64.9%), *Meloidogyne* (52%), *Hemicriconemoides* (37.8%), *Tylenchorhynchus* (24.3%), *Criconeoides* (16.9%), *Tylenchus* (15.5%), *Aphelenchus* (14.5%), *Hoplolaimus* (10.8%), *Rotylenchulus* (7.4%), *Paratrichodorus* (6.4%), *Pratylenchus* (6.1%), *Trichodorus* (5.7%), *Ditylenchus* and *Longidorus* (3.7%, each), *Zygotylenchus* (2.7%), *Xiphinema* (2%), *Aphelenchoides* (1.7%) and *Paratylenchus* (0.7%). Only *Helicotylenchus* and *Meloidogyne* were found on all the surveyed date palm cultivars (Al-Yahya *et al.*, 2001). A similar list of plant parasitic nematode associates was reported from date palms in Egypt, but with three species of *Meloidogyne* and five species of *Pratylenchus* observed, and differences in association levels with soil type and cultivar (Youssef and Lashein, 2013; Youssef, 2014). Lashein and Youssef (2013) further reported that root and soil counts of *Rotylenchulus reniformis* and *Helicotylenchus* sp. were correlated negatively with soil temperature and positively with soil moisture levels at different times of the year. A survey in the United Arab Emirates found nematodes associated with diseased date palms (Hashim, 1997). During field surveys of date diseases in coastal regions of Libya, root knot nematodes (*M. incognita* and *M. javanica*), root lesion nematodes (*P. penetrans* and *Pratylenchus* sp.), ring nematode (*Criconeoides* sp.) and others were associated with date palm rhizospheres (Edongali, 1996). A survey carried out in Algeria revealed the occurrence of five species of *Pratylenchus*. The most common species was *P. penetrans*, often associated with date palm (Troccoli *et al.*, 1992). *Criconeoides curvatus* and *Longidorus* sp. nov. were found on date palm in Florida (MacGowan, 1989). In Algeria, Lamberti *et al.* (1975) reported the occurrence of *P. penetrans* on date palm roots in the crescent of oases from Beni Ounif to Biskra, and there are reports of associations with species of *Hemicriconemoides*, *Xiphinema*, *Criconeoides*, *Trichodorus* and *Tylenchus*. *B. cocophilus* is also known to affect the date palm. A specimen in the Botanic Gardens, Trinidad, came down with red ring disease and produced a brownish ring. However, date palms growing in the main production areas prefer a hot dry environment that would limit the activities of the palm weevil, the vector of the red ring nematode, which thrives in areas of high humidity.

Management of date palm nematodes

Nematicides added to cultivated soil were screened for their ability to control soil nematodes. The higher the concentration, the higher the mortality rate. On date palms exhibiting Al-Wijam symptoms (i.e. stunting of leaves, yellow streaking of leaves and reduced size of fruit and fruit stalks), dazomet gave the best control, followed by carbofuran and oxamyl. *Longidorus* spp. were the most susceptible nematodes, followed by *Xiphinema*, but *Meloidogyne* were the most resistant species. However, there was no sign of recovery of treated date palm trees with Al-Wijam disease (Abdulsalam *et al.*, 1996). Youssef (2014) recommends starting and staying clean of nematodes by planting nematode-free date palm seedlings into clean soil (pre-treated with solarization and/or tillage). He further suggests using resistant or tolerant cultivars, disinfecting any nematode-infested seedlings with a suitable hot water treatment before planting, the application of poultry manure or oil cake amendments, as well as appropriate post-plant nematicides, weed control and the induction of innate resistance in susceptible date palm cultivars against root knot nematodes.

Arecanut

Arecanut or betel nut, *A. catechu*, occurs in the humid regions of Asia and the Malay islands. It is a masticatory of great antiquity, and betel chewing is a habit of nearly one-third of the world's population. The ripe fruits are sometimes used as an anthelmintic and astringent in Europe.

Nematodes of Arecanut

A number of nematodes have been reported from the rhizosphere of arecanut (see references in Griffith *et al.*, 2005), but only *R. similis* is known to be an important parasite of the palm. A number of other palms have been reported as hosts of *R. similis* (Table 14.2), and it would not be unexpected if nematode problems with some of these other palms became apparent in the years ahead.

Radopholus similis

The burrowing nematode *R. similis* was first reported from soil around roots of arecanut palm in Mysore, India, by Kumar *et al.* (1971).

Symptoms of damage

The most conspicuous symptoms of *R. similis* infestation are the appearance of lesions and rotting of roots. The nematode produces small, elongate, orange-coloured lesions on the young, succulent, creamy-white to light-orange coloured portion of the main and lateral roots. Subsequently, the adjoining lesions coalesce and cause extensive root rotting. The thick primary roots produced from the bole region of the palm exhibit large, oval, sunken, brown to black lesions, 2 mm to 2 cm in length (see Fig. 14.5).

Nematodes occur inter- and intracellularly in the cortex, but do not enter the stelar tissues. Large numbers of nematodes and their eggs are seen in the cavities that develop consequent to nematode feeding in the cortex (Sundararaju, 2000).

Biology and life cycle

The burrowing nematode takes 25–30 days to complete one life cycle (J2–J2) on arecanut seedlings at a temperature range of 21–31°C under glasshouse conditions. Chromosome studies have recorded the presence of a haploid number of chromosomes ($n = 4$) in many isolates of *R. similis* from arecanut roots (Koshy, 1986). The arecanut isolate of *R. similis* belongs to the banana race (Koshy and Sosamma, 1977) and multiplies well on carrot discs maintained on 1% water agar (Koshy and Sosamma, 1980).

Table 14.2. Palms reported as hosts of the burrowing nematode *Radopholus similis*.

<i>Archontophoenix cunninghamiana</i> (Seaforthia or Picabeen bungalow palm)
<i>Areca (Actinorhynchis) calapparia</i>
<i>Areca catechu</i> (betel-nut palm)
<i>Areca langlosiana</i>
<i>Areca macrocalyx</i>
<i>Areca normanbyii</i>
<i>Areca triandra</i>
<i>Chamaedorea cataractarum</i>
<i>Chamaedorea elegans</i> (parlour palm or Neanthebelia palm)
<i>Cocos nucifera</i> (coconut)
<i>Elaeis guineensis</i> (African oil palm)
<i>Phoenix canariensis</i> (Canary Island date palm)
<i>Phoenix dactylifera</i> (date palm)
<i>Rhapis excelsa</i> (large lady palm)
<i>Roystonea regia</i> (royal palm)
<i>Syagrus romanzoffiana</i> (queen palm)

The population densities of *R. similis* in arecanut fluctuate; maximum population occurs in roots during October to November and the minimum during March to June in India. Populations are also known to vary between samples, types of roots, palms, groves and soil types during the same period (Koshy and Sosamma, 1978a).

Disease complexes

The fungus *Cylindrocarpon obtusisporum* is found associated with lesions caused by *R. similis* in arecanut roots. The fungus, when introduced 3 weeks after nematode inoculation, caused more damage to plants compared with inoculations with the nematode alone, and it inhibited the rate of multiplication of the nematode (Sundararaju and Koshy, 1984, 1987).

Economic importance and population damage threshold levels

R. similis was recorded from 32% of root samples in the three major arecanut-growing states in south India, with a maximum population of 440 nematodes/g of root. *R. similis* was found in 55, 45, 44, 30 and 11% of root samples from plantations intercropped with banana, black pepper, cardamom, coconut and cacao, respectively, compared with 25% from plantations monocropped to arecanut (Griffith *et al.*, 2005).

The population damage threshold level on arecanut seedlings is 100 nematodes/seedling or 1/800 g of laterite soil. The percentage reduction of growth over uninoculated plants at this inoculum level can be 23, 39, 25, 19 and 38% relative to shoot length, shoot weight, girth at collar region, root length and root weight, respectively, under pot conditions in laterite soil.

Management

RESISTANCE/TOLERANCE: none of the 46 accessions of arecanut germplasm in the Central Plantation Crops Research Institute's (CPCRI, Kasargod, Kerala, India) germplasm collection is immune or highly resistant to *R. similis*. The cultivars Mangala (VTL-3) and Fiji (VTL-26) are highly susceptible, whereas the cultivars Singapore (VTL-17), Solomon Islands-2 (VTL-18c) and Saigon (VTL-27) are less susceptible to *R. similis*; cultivars Indonesia 6 (VTL-11), Mahuva 8 and Andaman-5 (VTL-29e) are tolerant to *R. similis*

(Koshy *et al.*, 1979; Sundararaju and Koshy, 1982). The cultivars Indonesia 6 (VTL-11) and Singapore (VTL-17) are known to yield 15% more nuts over local South Canara cultivar (Anon., 1974). Thus, these cultivars could probably be recommended for *R. similis*-infested areas. The hybrid VTL-11 × VTL-17 is highly resistant to *R. similis*.

CHEMICAL AND BIOLOGICAL: as arecanut is chewed directly by many consumers, the dosage, frequency and time of application of nematicides on arecanut have to be calculated carefully to avoid residues in the nut.

A pot culture experiment carried out under field conditions revealed that fensulfthion and aldicarb at 1 g a.i./seedling applied thrice a year for 3 consecutive years in pots gave control of *R. similis*, both in soil and in roots. Increases in plant growth with regard to shoot length, shoot weight, root length, root weight, number of leaves and collar girth with fensulfthion were 46, 168, 33, 173, 25 and 41%, respectively, over control plants after 3 years (Sundararaju and Koshy, 1986a). In a field experiment in India, treatment with fensulfthion at 50 g a.i./palm and aldicarb at 10 g a.i./palm applied during May/June, September/October and December/January for 5 years resulted in control of *R. similis* and a substantial increase in both number and weight of nuts compared with untreated palms (Sundararaju and Koshy, 1986b). However, the nuts were not analysed for their residues, if any, and the cost-benefit ratio has not been determined.

Field experiments were carried out in arecanut monocrop, arecanut + banana and arecanut + banana + pepper to evaluate the efficacy of neem oil cake and phorate singly and in combination for control of *R. similis* in the cropping system. Even though all the treatments were significantly superior to the untreated control, the best treatment in these experiments was 15 g of phorate in combination with 1 kg of neem oil cake, which controlled the *R. similis* population in arecanut and subsidiary crops very well (see references in Griffith *et al.*, 2005). A pot trial study to evaluate the combined effect of organic amendments and biocontrol agents, i.e. *P. lilacinum*, *P. penetrans* and arbuscular mycorrhizal fungi (AMF), against *R. similis* infecting arecanut (*A. catechu* var. Mangala) was conducted using sandy loam soil amended with various organic matter. The organic amendments used

were neem and marotti oil cakes, leaves and tender shoots of sunn hemp and *Gliricidia*, vermicompost, cow dung and coir pith. Maximum nematode control (95%) was recorded in soil amended with *Gliricidia* leaves and bioagents. Significant reduction in root lesion index and maximum leaf area was recorded in these plants. The percentage increase in height and root growth was best in plants grown in coir pith-amended soil with bioagents which was on a par with plants grown in soil enriched with *Gliricidia* leaves and bioagents. A decrease in nematode population was on a par in all treatments receiving organic amendments and bioagents, as well as bioagents alone compared with the nematode alone. All of the bioagents were re-isolated from all of the treated plants, even after 3 years. Although amendment of soil with organic matter in general was found to increase plant growth and reduce the nematode population, the differences were not significant compared with the introduction of bioagents in the absence of organic amendments (Koshy *et al.*, 1998b, 2002b).

Summary of management measures

The control of *R. similis* on arecanut is difficult under the high-density, multispecies, subsistence farming systems involving perennial crops such as coconut, banana, black pepper, betel vine, cardamom and cacao. The use of nematicides for control of the burrowing nematode on coconut or arecanut may cause problems of residual toxicity. The following control measures are suggested: (i) the use of nematode-free planting material of arecanut and other intercrops; (ii) avoiding *R. similis*-susceptible intercrops such as black pepper and banana in infested areas; (iii) the use of resistant/tolerant cultivars of arecanut, when available, and other crops in farming systems; (iv) the application of 5–10 kg/palm/year of green manure, preferably *Gliricidia* or *Crotalaria*; (v) the application of 1 kg of neem oil cake/palm/year; and (vi) the application of phorate at 3 g a.i./plant to the root zone of arecanut, banana and black pepper in June–July and October–November in arecanut-based farming systems (see references in Griffith *et al.*, 2005).

Methods of diagnosis

Soil and root samples for the detection of *R. similis* should be collected at a distance of 25–75 cm

from the bole of the palm at a depth of 25–75 cm when high population densities are present, such as during October/November in India. The method suggested for the extraction of *R. similis* from coconut roots can also be adopted for arecanut.

Other Palms

The reniform nematode, *R. reniformis*, is a sedentary, endoparasitic nematode that sometimes causes concern in field-grown or containerized palms for shipment in the USA. The concern is a regulatory issue rather than one of palm pathology. Most palms, except *Washingtonia robusta* and *Acoelorrhaphe wrightii* are non-hosts for the reniform nematode (Inserra *et al.*, 1994). These hosts, although suitable for nematode reproduction, are devoid of above-ground symptoms, suggesting tolerance. The regulatory problem arises because ornamental palms grow well in southern Florida conditions, where reniform nematode is a damaging pest to dicotyledenous field and vegetable crops. Ornamental palms that are contaminated or infested with reniform nematode are subject to quarantine in non-infested areas in Arizona, California and New Mexico, which grow valuable and susceptible crops such as cotton. *R. reniformis* has also been found in the rhizosphere of ornamental and certain date palm cultivars (Hayani, Zaghoor, Samani and Barhi) in Egypt (Ismail and Eissa, 1993; Youssef and Lashein, 2013).

Hot water treatments were evaluated to disinfect the roots and media of potted bamboo or reed palm *Chamaedorea seifrizii* and fishtail palm *Caryota mitis* of *R. similis*. A continuous hot water drenching (50°C for 15 min) of roots and media in pots or hot water dipping (50°C for 15 min) of bare-rooted plants were successful in eliminating all burrowing nematodes in bamboo palms. Fishtail palms were disinfected of burrowing nematodes after hot water drenching at 50°C for 13 min. Dipping plants intact in pots in a constant temperature water bath was not effective, as the root temperature remained below the thermal death point for nematodes, due to slow heat transfer. No evidence of thermal damage was observed in either palm species drenched with hot water at 50°C

for up to 20 min followed by hydro-cooling to ambient temperature. Ambient air cooling after heat treatment was detrimental, as the residual heat caused both vegetative and root damage in the potted palms. These air-cooled palms suffered reduced growth and required a longer recovery period (Tsang *et al.*, 2003).

General Conclusions and Future Activities on Nematodes of Palms

The foregoing has shown that fatal diseases in palms due to nematodes are unknown except for those that are attacked naturally by *B. cocophilus* and its chief insect vector, *R. palmarum*. The fact that red ring disease is at present confined to the New World restricts its economic importance to those palms that occur in the area, but others, such as the arecanut palm, are likely to be naturally susceptible, even in their areas of origin. Nematodes that have been recorded as pathogenic to palms in their areas of origin are only those that exist in the rhizosphere, such as *R. similis* of arecanut and coconut. This problem has not yet been recognized in the New World, but it is very possible that this and other nematode root problems on palms will become apparent in the years ahead. There is a danger of root nematodes, particularly *R. similis* and *R. reniformis*, being introduced through ornamental palms and other hosts.

The major concern of nematologists, plant pathologists and quarantine personnel, therefore, is to ensure against the possibility of red ring disease becoming more widespread, since the likelihood that other species of the palm weevil (e.g. other *Rhynchophorus* spp.) could be vectors to *B. cocophilus* in the Old World Tropics is quite strong. The palms of horticultural value are also susceptible, and could, in fact, increase the likelihood of the disease eventually moving out of Latin America in a palm where symptoms

are not so distinct and in which pathogenesis is prolonged. Indeed, there is every probability that symptomless carriers might exist, as in oil palms, that are slowly colonized by the nematode. Another problem occurs with the confusion of similar symptoms in other wilt diseases of palms that can hide the problem of a nematode until it is too late.

The International Bureau of Plant Genetic Resources has been helping in a number of coconut and other palm germplasm collection programmes, and some methods have been developed, such as embryo rescue for the introduction of clean coconut germplasm for research purposes. Koshy and Kumaran (1997) collected 1342 embryos of 15 accessions from the Indian Ocean islands for the first time. The commercial availability of synthetic, male-produced aggregation pheromones for the weevils that can vector red ring nematode allows for monitoring ports of entry for potential vectors, and recent advances in molecular diagnostic methods, such as loop-mediated isothermal amplification (LAMP) of *B. cocophilus* for detection in plant and insect tissue samples, should be helpful in early discovery and eradication attempts (Ide *et al.*, 2017). Continuing vigilance is required to thwart potentially new emerging plant pathogenic nematodes that might develop in association with coconut or other palm monocultures in the tropics and then be spread into other regions by human activities (Concibido *et al.*, 1984).

Generally, as crop plants for small farmers, cordon sanitaires are always necessary for vector-borne pathogens that can have fatal and cumulative effects on agroecosystems. Thus, control measures for palm diseases must always be inexpensive, effective and readily applicable in all economic circumstances. Essentially, of course, biological control measures and resistant cultivars should always be sought. The stability of the coconut agroecosystem favours management procedures with limited pesticide usage.

Notes

¹ <http://www.chemtica.com/>; <http://www.chemtica.com/site/?p=1770>; <http://www.chemtica.com/site/?p=1704>; <http://www.chemtica.com/site/?p=1699>; <http://www.chemtica.com/site/?p=2953>

² <http://www.chemtica.com/site/?p=1774>

³ <http://www.chemtica.com/site/?p=1770>

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15 Nematode Parasites of Coffee and Cocoa*

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Coffee

Coffee plants are perennial dicotyledonous trees or treelets of the Rubiaceae family, within the euasterid I clade of dicotyledonous plants. Formerly coffee belonged to the *Psilanthus* genus (20 species), but is now subsumed into *Coffea*, increasing the number of coffee species to 124 (Davis *et al.*, 2011). All species are diploids ($2n = 2 \times = 22$), except the cultivated allotetraploid *Coffea arabica* ($2n = 4 \times = 44$). Through phylogeographic analysis, Anthony *et al.* (2010) suggest the centre of origin of the former *Coffea* subgenus is in Lower Guinea, with a rapid and radial mode of speciation. Coffee typically occurs in tropical forest understories, with hard and dense wood and horizontal or near-horizontal paired plagiotropic branches carrying persistent leaves and hermaphrodite flowers organized by inflorescences paired and axillary (Davis *et al.*, 2006). *C. arabica* typically presents only one orthotropic stem, while they are multiple for *Coffea canephora*. Floral bud morphogenesis of coffee trees is induced by dry periods, with floral buds remaining dormant until the first rainfall. Flowering intensity depends on the time elapsed since the last flowering and on the quantity of rain inducing anthesis. Coffee blooming is important when rainfall exceeds

10 mm (Noirot *et al.*, 2016). Responses to dry spells and rainfall vary between coffee species and constitute the main reproductive barrier between coffee species. Indeed, the former *Coffea* subgenus is considered as a species complex in which interspecific gene flows are possible (Noirot *et al.*, 2016). All studied coffee species are able to intercross via controlled pollination, although with variable success rates, and many spontaneous interspecific coffee hybridizations have been observed in live collections or plantations, as is the case for the famous hybrids of Timor (*C. canephora* $\times$ *C. arabica*) founded in the homonymous island and which have been used largely for coffee leaf rust resistance gene introgression to susceptible *C. arabica* cultivars (Bertrand and Anthony, 2008). All species of the former *Coffea* subgenus are self-incompatible, except *C. arabica*, *Coffea heterocalyx* and *Coffea anthonyi*, while all species of the former subgenus *Psilanthus* are likely autogamous (Noirot *et al.*, 2016). Among the large number of *Coffea* species, only *C. arabica* (Arabica coffee) and *C. canephora* (Robusta coffee) are currently cultivated for beverage production, contributing 60% and 40% of world coffee production, respectively (ICO, 2016). A third species, *Coffea liberica* var. *liberica* (known as Liberian, Liberica or Excelsa coffee),

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is also cultivated for beverage, but with minimal production. Although *C. arabica* and *C. canephora* both originate from Africa, 85% of world Arabica coffee is produced by Latin America, with 44% from Brazil and 12% from Colombia; while 64% of world Robusta coffee is produced by Asia and Oceania, with 39% produced by Vietnam and 23% by Brazil.

The primary centre of diversity for *C. arabica* lies in the south-western Ethiopian highlands, the Boma Plateau of southern Sudan, and Mount Marsabit of northern Kenya. *C. arabica* is an amphidiploid species formed by hybridization between *Coffea eugenioides* and *C. canephora*, or ecotypes related to these two species (Lashermes *et al.*, 1999). All traditional Arabica cultivars (e.g. Caturra, Mundo Novo, Catuai) selected before the 1980s, and still predominantly cultivated, originated from two narrow genetic bases spread from Yemen – the primary centre of Arabica domestication – which are described as two distinct botanical varieties: *C. arabica* var. Arabica (usually called *C. arabica* var. Typica) and *C. arabica* var. Bourbon. The Typica genetic base originated from a single plant from Indonesia, while the Bourbon genetic base originated from a few coffee trees directly introduced from Mocha, Yemen, to Réunion Island (Anthony *et al.*, 2002). After the introduction and rapid expansion during the 1970s of coffee leaf rust disease (CLR) caused by the fungus *Hemileia vastatrix* in Latin America, many coffee-growing countries of this region initiated coffee breeding programmes, based on introgressing CLR resistance genes from accessions of Hybrid of Timor (Silva *et al.*, 2006) and leading to the creation of Catimor and Sarchimor cultivar families, characterized by CLR resistance (van der Vossen *et al.*, 2015). The genetic basis of Arabica cultivars, however, remains narrow, since a very low number of Timor Hybrid progenitors and traditional recurrent parents have been used for these introgression breeding programmes (Setotaw *et al.*, 2010 in van der Vossen, 2015). More recently, coffee breeding programmes have developed Hybrid F1 by crossing some cultivars with semi-wild Ethiopian accessions (van der Vossen *et al.*, 2015) in order to: (i) broaden the genetic basis of Arabica cultivars, including for resistance against aggressive root knot nematode (RKN) species; (ii) obtain strong hybrid vigour

or heterosis; (iii) maintain organoleptic quality; and (iv) rapidly create new cultivars within 5–7 years, compared to 20–25 years traditionally (van der Vossen *et al.*, 2015). *C. canephora* is considered as a metasppecies and presents a high genetic diversity (Cubry *et al.*, 2013). It has the greatest natural distribution across tropical Africa, being native to the lowland equatorial Congo River basin, tropical West and Central Africa, up to Lake Victoria at elevations of 250–1 500 m, with annual mean temperatures ranging from 22 to 26°C (Davis *et al.*, 2006). *C. canephora* is organized into two main groups: Congolese and Guinean, each subdivided into four and two subgroups, respectively. This diverse genetic resource is of great importance both for breeding purposes for Robusta as well as for rootstocks for Arabica.

Recently, some drastic changes in coffee production have been observed, related directly or indirectly with climatic change. Arabica production in Brazil increased steadily over the 10 years to 2014, but has since declined due to severe droughts following flowering, while for Robusta a similar scenario has played out, with rising production to 2015 and then drought and high temperatures affecting yields since. These abiotic stresses will exaggerate the impact of nematode attacks on coffee (Bertrand *et al.*, 2016). Additionally, and likely related with climatic anomalies, is an unprecedented epidemic of CLR between Colombia and Mexico in the north and Peru and Ecuador to the south (Avelino *et al.*, 2015).

Some physiological characteristics of coffee trees are important for cultural practices, and should be taken into account for nematode control and nematode-related field studies. First is that coffee berries are produced on second-year wood. The yield then depends on potential flowering nodes produced the previous year. Second, coffee trees have the unusual inability among woody perennials to shed excess fruit in relation to nutritional conditions, possibly due to its native shade habitat in the Ethiopian highland forests, with likely low flowering and therefore fructifications (see Campos and Villain, 2005). Coffee beans act as the priority physiological carbohydrate sinks during maturation, at the expense of roots, especially secondary roots that stop growing, or even die. Biotic stresses, therefore, such as nematodes or coffee leaf rust, and/or abiotic stresses that affect nutrition or photosynthesis

during a heavy fructification period, would have serious consequences on coffee tree survival.

Cultivation techniques

For a comprehensive overview of the main coffee cultivation techniques see Campos and Villain (2005), Vieira (2008) and Wintgens (2012), or alternatively the numerous technical manuals published by the coffee institutes of producing countries. Shading is not always necessary for cropping *C. arabica*, but agroforestry coffee cropping systems are very common in some areas, such as most of Central America and Mexico, where Arabica is grown mainly in mountainous areas characterized by a broken topography, frequently erodible volcanic soils and a hot dry season. In these environments, shade trees act as a climatic regulator and help to prevent soil erosion and contribute to improve soil characteristics (Bertrand *et al.*, 2016). Coffee production using agroforestry and intercropping systems, such as banana, citrus or macadamia trees, can improve sustainability by reducing input needs and increasing crop stability, enabling additional income and providing other ecosystemic services like carbon sequestration, water capture and groundwater supply, and soil conservation, as well as a biodiversity refuge, such as its role in trans-American bird migration (Gordon *et al.*, 2007; López-Gómez *et al.*, 2008; Hernández-Martínez *et al.*, 2009; Jha *et al.*, 2011). A wide range of tree species have been evaluated and are used as shade and wood providers in coffee cropping systems (López-Gómez *et al.*, 2008).

Nematode Parasites of Coffee

Numerous genera and species of nematodes have been associated with coffee worldwide, including some that are responsible for significant damage and/or plant mortality, resulting in important economic losses for coffee farmers and local economies. It is interesting to note, however, that on its continent of origin, Africa, damage to coffee by nematodes is not particularly well noted, due mostly to the limited attention that this crop has received. In contrast, coffee

faces dramatic damage from nematodes in both Latin America and Asia, where global coffee production is now concentrated. In Latin America, RKNs (*Meloidogyne* spp.) constitute the major nematode threat, while in Asia, lesion nematodes (*Pratylenchus* spp. and *Radopholus* spp.) are the most damaging and widely distributed on coffee (Campos and Villain, 2005; Souza, 2008; Trinh *et al.*, 2009a).

Meloidogyne

Meloidogyne spp. (RKNs) are globally the most widely distributed nematodes and likely the most harmful affecting coffee, especially in Latin America (Campos and Villain, 2005). To date, 19 *Meloidogyne* described species are reported parasitizing coffee (Campos and Villain, 2005; Carneiro and Cofcewicz, 2008; Humphreys-Pereira *et al.*, 2014) and can be separated into two categories: (i) widespread damaging species: *Meloidogyne exigua*, *Meloidogyne incognita* and *Meloidogyne paranaensis*; and (ii) the less widespread species, although some are highly pathogenic on coffee: *Meloidogyne africana* (= *Meloidogyne decalineata*, *Meloidogyne oteifai*), *Meloidogyne arabicida*, *Meloidogyne arenaria*, *Meloidogyne coffeicola*, *Meloidogyne enterolobii* (= *Meloidogyne mayaguensis*), *Meloidogyne hapla*, *Meloidogyne inornata*, *Meloidogyne izalcoensis*, *Meloidogyne javanica*, *Meloidogyne kikuyensis*, *Meloidogyne konaensis*, *Meloidogyne lopezi*, *Meloidogyne megadora* and *Meloidogyne thamesi*. It is important to note that this list of RKN species parasitizing coffee is likely incomplete, and is expected to increase as the use of reliable tools for identification, in combination with morphological observations, increases and improves (Carneiro and Cofcewicz, 2008; Correa *et al.*, 2013). Indeed, some species collected from coffee are yet to be described, as is the case for two populations from El Salvador, one from Vietnam and one from Tanzania (Carneiro *et al.*, 2004; P.Q. Trinh, Hanoi, Vietnam, 2017, personal communication; D.L. Coyne, Nairobi, 2017, personal communication). Due to previous misidentifications caused by overlapping morphological features and confusion, the distribution and relative importance of RKN species present on coffee remain uncertain (Carneiro and Cofcewicz, 2008; Janssen *et al.*, 2016, 2017).

Meloidogyne exigua*, *Meloidogyne incognita* and *Meloidogyne paranaensis

Distribution

These three RKN species are the most widely distributed on coffee. *M. exigua* and *M. paranaensis* have wide American distribution, while *M. incognita* is highly cosmopolitan. They also appear to be present over most of the altitudinal and latitudinal range of coffee growing in Latin America (Campos and Villain, 2005; Villain *et al.*, 2013; López-Lima *et al.*, 2015). As highlighted by Elling (2013), *M. exigua* and *M. paranaensis* are considered as minor species, but are in fact emerging as major tropical and subtropical crop problems, as evidenced for coffee.

M. exigua is the most widely distributed RKN on Arabica coffee in the Caribbean, Central and South America, with a presence in all major coffee-growing countries between Honduras to Parana State in Brazil (Campos and Villain, 2005; Carneiro and Cofcewicz, 2008; Villain *et al.*, 2013). On coffee, however, this RKN is almost exclusively a parasite of *C. arabica*, since most *C. canephora* germplasm shows complete resistance to *M. exigua*, controlled by a major gene, *Mex-1*, which was transferred to most introgressed cultivars of Catimor and Sarchimor varietal families (Noir *et al.*, 2003). *M. exigua* has been detected in primary natural forest areas: the Atlantic forest in Rio de Janeiro State (Lima *et al.*, 2005) and the Amazon forest in the northern region of Mato Grosso State (Silva *et al.*, 2008) in Brazil, as well as in Costa Rica (López and Vilchez, 1991) and in Martinique (Quénéhervé *et al.*, 2011). All these observations indicate that *M. exigua* is a native species of Latin America and probably with an original wide distribution. However, *M. exigua*, together with *M. coffeicola*, has recently been confirmed from Vietnam and surprisingly from *C. canephora* (Trinh *et al.*, 2013).

The real distribution of *M. incognita* on coffee remains uncertain, since numerous previous reports of putative *M. incognita* populations could likely have been misidentified through using perineal patterns, which do not discriminate from some species, such as *M. paranaensis* or the recently described *M. izalcoensis* (Campos and Villain, 2005; Carneiro and Cofcewicz, 2008; Villain *et al.*, 2013). Currently, the only country in Latin America where a wide distribution of

M. incognita on coffee may be seriously considered is Brazil, because of the numerous reliable identifications based on species-specific sequence characterized amplified region (SCAR) markers and/or esterase phenotypes (Campos and Villain, 2005; Carneiro and Cofcewicz, 2008). The presence of *M. incognita* is confirmed from Nicaragua (Herrera *et al.*, 2011b) and Costa Rica (Villain *et al.*, 2013). However, in both countries, *M. incognita* seems to have a punctuate distribution on coffee (both *C. arabica* and *C. canephora*), contrary to *M. exigua*, which appears as the most widespread RKN on coffee. In Vietnam, *M. incognita* is widely distributed on *C. canephora* in the Western Highlands and has also been detected on *C. arabica* in Daklak province (Trinh *et al.*, 2013). It is also confirmed from coffee in Tanzania and Uganda (R.M.D.G. Carneiro *et al.*, unpublished).

M. paranaensis, described by Carneiro *et al.* (1996) from a coffee plantation in Paraná State, Brazil, has a disjointed and an exclusively American distribution, with a presence in Brazil, Peru, Guatemala, Hawaii, (Campos and Villain, 2005) and Mexico (López-Lima *et al.*, 2015). This fragmented distribution could be related to early coffee seedling introductions, but also by banana introductions, since it is also a good host for *M. paranaensis* (Villain *et al.*, 2013; López-Lima *et al.*, 2015). The range of *M. paranaensis* is constantly extending, at least inside the countries where it is already present, by propagation material or soil transport. Latterly, many reports of *M. paranaensis* are from Brazil, such as in the northern region of São Paulo State, numerous localities of Minas Gerais, the first Brazilian state growing *C. arabica*, with over half of the national production (Castro and Campos, 2004; Salgado *et al.*, 2015), in Espírito Santo State on *C. canephora* crop (Barros *et al.*, 2011, 2014) and in Paraná and Goiás States (Silva *et al.*, 2009). In Guatemala, where *M. paranaensis* seems to be the predominant RKN species (Villain *et al.*, 2013), corky root symptoms similar to those observed in cases of coffee root parasitism by *M. paranaensis* were already being observed at the beginning of the 20th century (Alvarado, 1935), indicating an age-old threat to coffee in Guatemala. *M. paranaensis* was detected in primary forest in Martinique (Quénéhervé, 2009; Quénéhervé and Van den Berg, 2009; Quénéhervé *et al.*, 2011), which is coincidentally the locality for

the first introduction of *C. arabica* into the Americas, during the 18th century (Mauro, 2014).

Symptoms of damage

The root symptoms caused by the RKN species on coffee can differ between species, but also according to the age of the root for the most aggressive RKN. Root symptoms should not be used to diagnose species, only to indicate evidence of RKN infection and as a possible indication of species (Muniz *et al.*, 2009; Villain *et al.*, 2013).

M. exigua generally causes numerous small to large, rounded or elongated galls, mostly on new, whitish roots (Fig. 15.1). Each gall usually contains several females and egg masses, which are produced into the cortex and generally are not exposed. The galls are initially white to yellowish brown, and turn dark brown as the root ages. Necrotic areas can appear on old galls and may be aggravated by secondary infections by fungi and bacteria.

M. exigua alters the physiological state of the coffee plant by affecting water and nutrient translocation (Barbosa *et al.*, 2004, 2010; Rezende *et al.*, 2013). Galls caused by *M. exigua* are mainly distributed on the new, whitish roots and remain intact for a long period without cracking or splitting the roots. Necrosis on older galled roots is somewhat compensated by the high rate of production of feeder (fine) roots. Damage to the plants, therefore, depends mainly on the physiological state of the plant, and thus on agroecological conditions. However, significant production losses due to *M. exigua* have been reported from commercial coffee plantations that use modern and advanced technologies, correct fertilizer use and integrated pest management (IPM) practices (Barbosa *et al.*, 2004). The production of young (<5 years) coffee trees decreased by 13–30%, while 45% of production losses were observed for older plantations. Rezende *et al.* (2013) observed a 23% productivity reduction of susceptible, compared to resistant, cultivars. In Costa Rica, production losses caused by *M. exigua* on susceptible *C. arabica* cv. Caturra were assessed as 8–16%, with no detection of foliar nutrient level alteration, indicating a likely impact of the nematode mostly on water uptake (Rojas and Salazar, 2014). Similarly, young coffee plants were the most susceptible to *M. exigua* during the dry season, with reduced growth, defoliation and some mortality observed (Barbosa *et al.*, 2004; Muniz *et al.*, 2009).

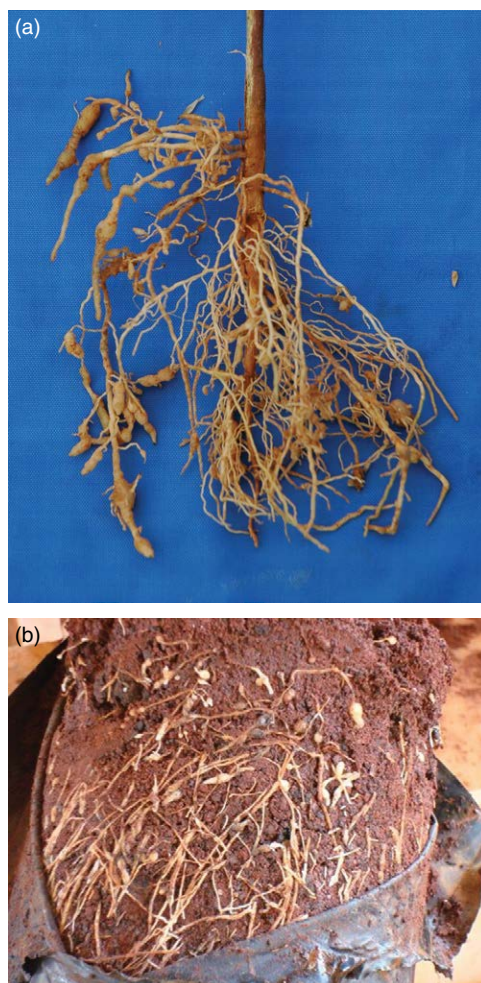


Fig. 15.1. Six-month-old *Coffea arabica* seedling roots in Brazil parasitized by *Meloidogyne exigua*: (a) roots with numerous rounded or elongated galls (photograph courtesy of L. Villain); (b) numerous root galls visible at the surface of a nursery bag (photograph courtesy of S. Salgado).

In Brazil, *M. incognita* causes peeling and cracking of the root surface due to hypertrophy of infected tissues, in addition to disruptions of the cortex and epidermis caused by protruding egg masses breaking the root surface. Dark spots along the root indicate where the females are located. Sometimes, root swellings rather than galls are observed on lateral roots. These cortical tissue disruptions turn necrotic, leading to root death (Fig. 15.2). Eventually, the plant responds to infection by forming numerous new and superficial absorbing roots (Fig. 15.3).

The infected plants show foliar chlorosis, defoliation, general decline and reduced growth, leading sometimes to plant death (Fig. 15.4).

Infection by *M. paranaensis* results in very impressive symptoms, especially on woody roots from the nursery stage (Fig. 15.5). Such symptoms have been observed in Brazil, Guatemala and Mexico. On younger roots, elongated small galls and root swellings are apparent. In older and woody roots, moderate to huge swellings turn corky and show cracking over time. In Latin America, this is referred to as ‘corchosis’ (corky root disease) of coffee. These symptoms



Fig. 15.2. *Coffea canephora* roots in Vietnam infected with *Meloidogyne incognita*, causing corky root swelling symptoms, turning necrotic. Necrosis caused by *Pratylenchus coffeae* additionally visible on fine young roots. (Photograph courtesy of P.Q. Trinh.)

can affect all roots, including the taproot, eventually up to the collar, which ultimately leads to the complete destruction of the root system (Carneiro and Cofcewicz, 2008; Villain *et al.*, 2013; López-Lima *et al.*, 2015). Above-ground, damage results first in chlorosis and nutritional deficiency symptoms, defoliation (Fig. 15.6), then a progressive decay of the plants for both *C. arabica* and *C. canephora* (Barros *et al.*, 2011; Villain *et al.*, 2013; López-Lima *et al.*, 2015).

In new coffee plantations, where abiotic stresses are intense, due to low altitude with high temperatures for *C. arabica*, low-density shade cropping, extended drought, low fertility and/or low pH soils, high plant mortality rates are observed before the infected plants begin to produce in the second or third year. When agroecological conditions are favourable for coffee, the decay process of coffee trees escalates mainly when the plants begin to produce (Villain *et al.*, 2002; López-Lima *et al.*, 2015).

Biology and life cycle

M. incognita and *M. paranaensis* reproduce by mitotic parthenogenesis, while *M. exigua* reproduces by meiotic parthenogenesis (Carneiro and Cofcewicz, 2008; Castagnone-Sereno *et al.*, 2013). At 25–30°C, *M. exigua* has a life cycle of 32–42 days (Lima and Ferraz, 1985). At 28°C, *M. paranaensis* and *M. exigua* exhibited a 45- and 38-day life cycle duration, respectively, on susceptible *C. arabica* cv. Mundo Novo (Guimarães, 2016). The life cycle of *M. incognita* on *C. arabica*



Fig. 15.3. *Coffea arabica* roots in Brazil infected with *Meloidogyne incognita*, exhibiting corky root swelling symptoms on coarse roots and a profusion of new fine roots. (Photograph courtesy of S. Salgado.)



Fig. 15.4. Severe decay (defoliation, branch death, low production) of *Coffea arabica* trees infected with *Meloidogyne incognita* in Brazil. (Photograph courtesy of S. Salgado.)



Fig. 15.5. Typical corky root symptoms in Guatemala caused by *Meloidogyne paranaensis* on taproot and coarse roots of: (a) *Coffea arabica*; and (b) *Coffea canephora*. (Photograph courtesy of L. Villain.)



Fig. 15.6. Symptoms of chlorosis due to *Meloidogyne paranaensis* in the field: (a) *Coffea arabica* in Brazil; (b) *C. arabica* in Veracruz State, Mexico; (c) *Coffea canephora* in Guatemala. (Photographs courtesy of S. Salgado and L. Villain.)

cv. Mundo Novo was completed in 48, 40, 32 and 32 days at 20, 24, 28 and 32°C, respectively (Jaehn, 1990).

Pathotypes, races or biotypes

Races have been established for coffee-parasitizing populations of *M. incognita* (4 races) and *M. exigua* (2 races) according to their capacity to reproduce on some differential hosts. However, controversy exists about the concept and stability of *Meloidogyne* spp. races. Actually, no correlation could be found between cytogenetic, isoenzymatic or molecular intraspecific polymorphism and host races (Castagnone-Sereno *et al.*, 2013). It has also been difficult to correlate some of these phenotypic or genetic characters with differences of pathogenicity observed in the field or under controlled conditions. Carneiro *et al.* (2000) differentiated three races of *M. exigua* by their efficiency to reproduce on some hosts: race 1 (pepper and coffee), race 2 (tomato, pepper and coffee) and race 3 (rubber tree). Four esterase phenotypes (E1, E2, E2a, E3) and three malate dehydrogenase (Mdh) phenotypes (N1, N1a, N2) have been described for 16 populations of *M. exigua*, but these enzymatic phenotypes do not separate *M. exigua* races (Muniz *et al.*, 2008). According to the same authors, phylogenetic analyses showed high intraspecific polymorphism (25.9–59.6%) among the 16 *M. exigua* populations studied, which all clustered closely together with 100% bootstrap support. One population of *M. exigua* from Rio

de Janeiro State has been described as highly virulent on *C. arabica* cv. IAPAR 59 (Sarchimor) bearing the resistance gene to *M. exigua*, *Mex-1* (Muniz *et al.*, 2009). Four races of *M. incognita* are known to occur in Paraná and São Paulo States of Brazil, (da Costa *et al.*, 1991; Carneiro *et al.*, 1992). In Paraná State, race 2 predominates (Carneiro *et al.*, 1990), while in São Paulo State, race 1 predominates (Monteiro *et al.*, 1995; de Oliveira *et al.*, 2000, 2001; Lordello, 2002). A shift from race 2 to race 4 has been observed with an *M. incognita* population collected from coffee 5 years after consecutive rearing on tomato (Carneiro, 2015). Three esterase phenotypes of *M. paranaensis* have been described: P1, P2 and P2a. P1 has been detected in Hawaii (one population), Guatemala (one population) and in Brazil, where it seems to be predominant; P2 is present in Brazil, while P2a has been detected only in Guatemala (Carneiro *et al.*, 2004; Santos *et al.*, 2017b). The same authors reported low genetic variability for this species, although they observed that the populations from Guatemala (P2a) diverged from all other P1 and P2 populations from Brazil, Hawaii or Guatemala. Santos *et al.* (2017b) additionally showed different levels of aggressiveness between *M. paranaensis* populations on susceptible *C. arabica* cultivars but without relation with their esterase phenotype. It was also demonstrated in this study that *C. arabica* and *C. canephora* cultivars, previously identified as resistant to *M. paranaensis*, remained resistant to all the studied populations.

Survival and means of dissemination

The main ways coffee nematodes are disseminated include: (i) use of infested soil in nurseries without disinfection; (ii) planting out infected coffee seedlings from the nursery; and (iii) planting infected plants of associated host crops, especially banana for *M. paranaensis* and *M. incognita* (Villain *et al.*, 2013; López-Lima *et al.*, 2015). In Minas Gerais State, Brazil, a state government law has, since 1996, enforced farmers to certify the absence of any *Meloidogyne* species in coffee seedling roots from an authorised nematology laboratory. This tactic has reduced the spread of *Meloidogyne* species within the state and avoided the introduction of infected seedlings into non-infested areas. Unfortunately, such prophylactic measures are uncommon elsewhere.

Nematode survival is influenced by multiple environmental factors that interfere directly with their parasitic capacity. Changes in the corporal lipid content of *M. exigua* infective juveniles (J2) are correlated with temperature, rainfall and J2 soil density (Rocha *et al.*, 2010). Moreover, J2 infectivity and reproduction on tomato were correlated with their lipid reserves. In the absence of a host, *M. exigua* does not survive in soil for more than 6 months (de Moraes and Lordello, 1977). *M. coffeicola* also shows low persistence in the soil (Rebel *et al.*, 1976; Carneiro Filho and Yamaguchi, 1995). *M. coffeicola* also appears to have a low capacity to infect coffee seedlings or young trees. Consequently, no success has been observed with artificially inoculating coffee seedlings. On the contrary, high infection levels of coffee by *M. incognita* have been observed in infested soils that have been fallowed without host plants for 6 months, with just a 27% reduction from the initial density (Jaehn and Rebel, 1984).

Environmental factors affecting parasitism

Suppression or reduction of nematode densities in the field occurs naturally due to physical factors, organic matter and soil microbiota. When *M. incognita* and *M. paranaensis*, collected from coffee roots, were reared on tomato for 2 years consecutively and inoculated back on to coffee,

these populations were no longer pathogenic on coffee (Carneiro and Jorge, 2001). Coppicing of coffee trees is a common and cyclic practice used by coffee growers for rejuvenating the plants and maintaining productivity. However, coppicing exacerbates the damage caused by *M. incognita*, *M. paranaensis* or *M. exigua*, since it induces death of the fine absorbing roots, increasing the proportion of nematodes versus the number of viable roots (Gonçalves and Silvarolla, 2001).

With an increase in air temperature and rainfall, and during new root flushes, population densities of *M. exigua* rise (Souza *et al.*, 2008). In Brazil, the high temperatures at the end of spring and during summer (from September to January), together with the new root flushes, induce *M. exigua* reproduction and oviposition (Costa *et al.*, 2012). Thus, later sampling during the dry season provides a more accurate assessment of *M. exigua* J2 field densities.

Soil, weather, topography and crop management variously influence nematode dynamics. Low *M. exigua* population densities occurred in soils with low sand and high Zn and K contents in Costa Rica, while the opposite was seen for *Pratylenchus coffeae*; *M. exigua* occurred mainly at lower altitudes compared with *P. coffeae* (Avelino *et al.*, 2009). Organic matter contents above 12% dry mass affect RKNs negatively (Avelino *et al.*, 2009). Rhizobacteria influence *M. exigua* in Brazilian coffee plantations (Oliveira *et al.*, 2007); several rhizobacteria strains obtained from coffee plants in Minas Gerais State, Brazil, significantly reduced *M. exigua* densities, especially *Bacillus megaterium* (strain 54-06).

Climatic anomalies such as prolonged droughts, unusually high temperatures or prolonged rainy periods can affect plant parasitic nematodes and/or their impact on crops (Bertrand *et al.*, 2016). Global warming will effectively shorten the life cycle of many tropical species parasitizing coffee, promoting population increases and extending their geographical range, as predicted for *M. incognita* in Brazil, particularly on coffee (Ghini *et al.*, 2008, 2011). With increases in temperature and CO₂ concentration, carbon allocation in plants will change, resulting in greater carbon concentration in roots, which will likely promote nematode

reproduction and impact, particularly in perennial crops such as coffee (Bertrand *et al.*, 2016). The impact of nematodes on crops under climate change will be experienced through the development and spread of other pests and diseases, whose combined damage will lead to an overall greater impact; for example, the CLR regional epidemic, likely because of regional climate anomalies (Avelino *et al.*, 2015). It has been observed that the combination of both CLR and nematodes has been lethal for coffee trees. Under abiotic stress conditions, nematodes, which reproduce well on coffee but do not usually represent a major threat when plantations are under optimum conditions and well managed, may become a serious stress for the crop, since they alter water and nutrient translocation, as with *M. exigua* (Bertrand *et al.*, 2016).

Other hosts

In Guatemala, *Impatiens balsamina*, a common weed in coffee plantations, is a good host of *M. paranaensis* (Campos and Villain, 2005; Villain *et al.*, 2013). *M. paranaensis* reproduces well on *Cyperus rotundus*, *Solanum americanum*, *Raphanus raphanistrum*, *Sorghum halepense*, *Galinsoga ciliata*, *Eleusine indica*, *Ilex paraguariensis*, *Ageratum conyzoides*, *Emilia sonchifolia*, *Ipomoea grandifolia* and *Echinochloa colona* (Santiago *et al.*, 2000; Roese, 2003; Roese and Oliveira, 2004). Soybean is also a good host for *M. paranaensis* (Castro *et al.*, 2003), as are the Andean fruit crops *Cyphomandra betacea* (syn. *Solanum betaceum* or tree tomato) and *Passiflora ligularis* (sweet granadilla) in Colombia (Munera-Urbe, 2008).

In Brazil, *Chenopodium album*, *Alternanthera tenella*, *E. colona*, *Amaranthus hybridus*, *Caperonia palustris*, *Ipomoea nil*, *Polygonum persicaria*, *Eclipta alba*, *Verbena litoralis*, *A. conyzoides* and *Galinsoga parviflora* are viewed as good hosts for *M. incognita* races 1 and 3, and also for *M. javanica* and *M. paranaensis* (Mônaco *et al.*, 2009).

Some crop hosts of *M. exigua* include rubber, *Hevea brasiliensis* (Santos *et al.*, 1992), *Grevillea robusta* (Santos, 1988), watermelon and onion (de Moraes *et al.*, 1972, 1973) and pepper (Lordello, 1964), as well as weeds that are commonly found in coffee plantations: *Solanum nigrum*, *Ipomoea aristolochiaefolia*,

Stachys arvensis, *Leonurus sibiricus*, *Amaranthus deflexus*, *G. parviflora*, *Euphorbia heterophylla*, *Taraxacum officinale* (Curi, 1973; Lima *et al.*, 1985) and *Citrullus lanatus* (= *Citrullus vulgaris*) (Ponte, 1977). For *I. indica* var. *acuminata*, *S. arvensis* and *L. sibiricus*, the reproduction of *M. exigua* was higher than on *C. arabica* var. Mundo Novo (Lima *et al.*, 1985). In Colombia, *Commelina diffusa*, *Hydrocotyle* sp., *S. nigrum*, *Inga* sp. and *C. rotundus* are hosts of *M. exigua* (Aragon *et al.*, 1978). Cocoa is a host of *M. exigua* in Bolivia (Bridge *et al.*, 1982). *Miconia* sp., a tree found in a virgin forest in Juntas de Pacuar, Perez Zeledon county, and *Spananthe paniculata* (weed type) are hosts of *M. exigua* in Costa Rica (López and Vilchez, 1991).

M. incognita is well known as having an extremely wide host range, infecting many crops, weeds and ornamental plants (Perry *et al.*, 2009). In Nicaragua, *Desmodium heterocarpon* subsp. *ovalifolium* suppressed *M. incognita* (Herrera and Marban-Mendoza, 1999).

Disease complexes

The fungus *Rhizoctonia solani*, inoculated around plants of *C. arabica* or *C. canephora* following *M. exigua* infection, caused more root necrosis and defoliation than when both pathogens were inoculated either simultaneously or separately in the greenhouse (Souza, 1977). Isolations from galled roots and histopathological studies 85 and 115 days after the inoculation of nematode-infected plants with *R. solani* revealed extensive fungal colonization within the coffee root systems. The fungus *Fusarium oxysporum* f.sp. *coffea*, inoculated on to coffee seedlings 4 weeks after *M. incognita*, increased chlorosis, root necrosis, wilting and stunting. Fungal hyphae were observed in giant cells and xylem vessels (Negron and Acosta, 1989). In Veracruz State, Mexico, López-Lima *et al.* (2017, unpublished results) observed the presence of *F. oxysporum* associated with *M. paranaensis* in all internal tissues of sampled coffee corky roots.

Economic importance

Plant parasitic nematodes are estimated to cause between 10 and 20% coffee losses worldwide

(Abd-Elgawad and Askary, 2015). However, for perennial crops, estimating economic losses is difficult since infection occurs over a long period and directly affects not only production but also the cost of replanting following plant death. Production losses due to RKNs, however, depend on the species present, the population density and field and agroecological conditions (Villain *et al.*, 2002). This could explain the variation in estimates of production losses caused by *M. exigua* between studies and countries, such as that observed between Brazil and Costa Rica (Barbosa *et al.*, 2004; Rezende *et al.*, 2013; Rojas and Salazar, 2014). In Costa Rica, infection by *M. exigua* causes general weakening of the trees, with an estimated drop in yields ranging from 10 to 20% (Bertrand *et al.*, 1997). In Colombia, economic losses of coffee due to *M. exigua* and *M. javanica* have been estimated at US\$800 million/year (Barriga, 1976).

Over the years, the relative importance of the major coffee RKN species has shifted in Brazil. From 1975 to 1990, *M. incognita* affected the best coffee-growing areas, especially in north Paraná and west São Paulo States, resulting in the complete destruction of entire plantations, forcing farmers to switch crops and leading Lordello (1984) to declare *M. incognita* as 'a disaster pathogen becoming the worst enemy of coffee in some areas of São Paulo State'. However, some of this damage was due to *M. paranaensis*, but was previously described as *M. incognita* race 5. More recently, up to 50% of decline and dieback of coffee trees, along with yield suppression, are associated with *M. paranaensis* in Brazil (Moens *et al.*, 2009). It has recently spread quickly in Minas Gerais and now affects many coffee plantations of this State (Castro *et al.*, 2008, Salgado *et al.*, 2015). *M. paranaensis* is now considered the RKN most harmful to coffee in Minas Gerais State, because of the severe root damage, leading to rapid plant mortality. In the traditional Arabica coffee-growing region of Alta Paulista in north São Paulo State, replanted Arabica cultivars must now be grafted on to *C. canephora* cv. Apoatã rootstock, which is resistant to *M. incognita* and *M. paranaensis* and immune to *M. exigua*, in order to continue cropping coffee in this region. *M. paranaensis* is emerging as a serious threat to *C. canephora* cv. Conilon in Espírito Santo State (Barros *et al.*, 2014). Even though *M. incognita*

and *M. paranaensis* are more pathogenic on coffee than *M. exigua*, *M. exigua* is more likely responsible for greater losses to coffee in Brazil, due to its wide distribution in the most traditional and competitive coffee-producing states, such as São Paulo and Minas Gerais (Gonçalves and Silvarolla, 2001).

In Guatemala, no statistics are available on economic losses caused by *M. paranaensis* on coffee (the principal coffee RKN species in Guatemala), although this species is responsible for the entire destruction of coffee plantations in the south-western region of Sierra Madre. As in Brazil, many coffee growers have had to replant *C. arabica* cultivars grafted on to resistant rootstock *C. canephora* cv. Nemaya, which is provided by the National Association of Coffee (Villain *et al.*, 2008, 2013). *M. paranaensis* has also been observed causing serious damage to commercial *C. canephora* plantations (a marginal crop in Guatemala), with a progressive decay beginning with chlorosis and production loss. On pruning the decayed plants, most fail to regenerate, and eventually die (Villain *et al.*, 2013).

In Mexico, the coffee corky root disease associated with *M. paranaensis* is now considered a severe threat to coffee production, causing up to 35% of the annual *C. arabica* plant losses in the two highest coffee-producing states, Veracruz and Chiapas (López-Lima *et al.*, 2015). Although Mexico has an important *C. canephora* cv. Robusta industry for soluble (instant) coffee production, *M. paranaensis* has not been reported on this crop, even though some Robusta cropping areas are situated close to Arabica sites.

Control measures

The control of nematodes in a perennial crop is more difficult than in annual or herbaceous crops, due to difficulties in eliminating or reducing it after it has become established. The long-term nature of perennial crops makes rotation schemes difficult to implement. With perennial crops, nematodes that survive the control practices have time to recover and build up to destructive levels. Moreover, old roots or plants remaining in the field, weeds or other crop hosts provide sources of nematode re-infestations, negating the beneficial effects of control practices (Campos and Villain, 2005).

EXCLUSION: the first and main measure for handling these highly pathogenic RKNs is prophylaxis or prevention, effectively avoiding the dissemination of these nematodes through infected seedlings or soil (Salgado and Rezende, 2010). Agricultural tools should be washed thoroughly before moving to a new field. Water runoff from infested plots should be prevented by trenches and canals and tight hedges planted (Dhanam *et al.*, 2011). Often, phytosanitary inspections of commercial coffee seedling nurseries remain weak or non-existent, but when enforced can make a big difference, such as in Minas Gerais State, where coffee seedlings are certified by law for the absence of *Meloidogyne* spp. In Brazil, the Agriculture Department has implemented regulations for the production and commercialization of coffee seedlings since 2012, with regard to *Meloidogyne* spp., but this now needs to be revised to include *Pratylenchus* spp. Problems arise, however, where coffee growers produce their own seedlings without taking nematode control into account (Fig. 15.7).

The production of RKN-free seedlings has generally relied on using nematode-free soil and irrigation water, including the use of methyl bromide to sterilize soil. However, with the removal of methyl bromide, solarization or steam is used to disinfect soil. In Brazil, experiments with solarization eliminated *Meloidogyne* spp. in nursery soil using solar collectors for 2 days (Ghini, 2004). The water source for nursery irrigation needs to be selected carefully, avoiding dams in which runoff water originates from hillsides cultivated with infected coffee. Seedlings

infected with RKNs should be destroyed (burned) and under no circumstances planted into new fields (Campos and Villain, 2005).

CHEMICALS: numerous nematicides have been used effectively against nematode pests of various annual crops, but there has been poor progress made in the control of nematodes in perennial crops (Brand *et al.*, 2010). In order to control coffee nematodes efficiently, nematicides must be systemic. The systemic insecticides, the organophosphates and organocarbamates, that have potential for nematode control are rarely phytotoxic at concentrations used for field control. Brand *et al.* (2010) report that fumigants such as chloropicrin, methyl isothiocyanate (Dazomet) and metasodium have good activity against nematodes. The major disadvantages are that they are water dispersed. Nematicidal activity is usually confined to a shallow root zone or rhizosphere, and is often a result of narcotization and nematode behaviour modification rather than killing. However, disruption of nematode infection and their development and reproduction can temporarily slow or halt increases in nematode density. The application of nematicides on coffee infected with *M. incognita* or *M. paranaensis* has not been recommended, due to the rapid destruction of large parts of the root system by the nematode. For *Meloidogyne* species causing similar symptoms to those of *M. paranaensis*, *M. incognita* and *M. coffeicola*, the use of nematicides as a control measure is not recommended, due to the destruction of large portions of the plant root system by the nematode (Campos and Silva, 2008). *M. coffeicola* tends to occur in older plantations that require renovation rather than treatment. The application of nematicides depends on the plant condition, the nematode species and population densities. In addition, the use of some chemical products is restricted by coffee production certification norms. Some coffee certifications contribute indirectly to productivity increases by promoting better cropping practices (van Rijsbergen *et al.*, 2016).

Many granular nematicides are effective in decreasing nematode densities of *Meloidogyne* species up to 3 months after application in plants with typical root galls, such as *M. exigua* (Campos and Silva, 2008), after which population densities may increase. *M. exigua*-infected coffee treated for 5 consecutive years with nematicides



Fig. 15.7. *Coffea arabica* seedlings from Veracruz State, Mexico, infected with *Meloidogyne paranaensis* due to the use of untreated, infested potting soil. (Photograph courtesy of L. Villain.)

produced 30.9% higher yields than non-treated coffee. However, the nematicide does not eradicate the nematode. The fluoroalkenyl fluensulfone has a greater nematocidal activity against *Meloidogyne* spp. than against migratory nematodes, as demonstrated *in vitro* and in soil naturally infested with *P. penetrans* (7.4 nematodes/g soil) from a pepper field (Oka, 2014); at very low concentrations, fluensulfone caused irreversible effects on *Meloidogyne* spp. In Brazil, fluensulfone (MCW 2), commercialized as Nimitz® (ADAMA), has been recommended for the control of *M. exigua* in coffee plantations, applied in strips of 50 cm either side of the plants, following the clearance of litter under the coffee. Almeida *et al.* (2015) observed efficient control of *M. exigua* in plantations up to 90 days after application, using dosages of fluensulfone at ≥ 2.0 l/ha. The study did not continue longer than the 90-day period, and further studies are necessary to assess the duration of effect as well as to elucidate the mode of action and determine whether fluensulfone is a 'true' nematicide affecting nematode metabolism prior to death (Kearn *et al.*, 2014). Santinato *et al.* (2016) verified the effect of fluensulfone (Nimitz®) via drench at 2.0 l/ha for the control of *M. paranaensis* in new and recently pruned plantations.

Nematicides can reduce or nearly cancel nematode multiplication in the field for up to 90 days, such as the organophosphorus cadusafos (Rugby®, FMC) applied at 200 g/l against *M. exigua* (Campos and Silva, 2008). The application of granular nematicides needs to be incorporated into the soil underneath the foliage, toward the stem, which requires specialized equipment. Rugby also needs to be applied following litter clearance, which again requires an adapted superficial soil cleaning device with the application machine, creating further expense. The timing of application can also be important, such as for granular products, which require water to liberate activity and therefore need to be applied at the beginning of the rains (Campos *et al.*, 1985).

There is, however, only limited research (at least in Brazil) regarding new nematicides against nematodes in coffee plantations. Chalcones and acetophenones have considerable toxicological activity against *M. incognita*, which could potentially serve as management options for coffee. Further to studies conducted *in vitro*, Caboni *et al.* (2016) indicated alternative modes

of action, lower toxicity, improved selectivity and generally greater environmental acceptability.

However, there is a general consensus that more environment-sensitive, biologically based options for nematode management should be explored and exploited, such as building on the biodiversity and natural mechanisms (antagonisms, synergies, etc.) of agroecosystems. With the development of increasingly efficient molecular tools such as metagenomic techniques, understanding the importance and eliciting the important components of the microbiome and microbiota of the rhizosphere and host plant becomes increasingly possible. For example, identifying the physiological functions and genetic triggers for defence mechanisms to abiotic or biotic stresses, and how these may be affected by chemical pesticides. The potential for developing control measures through a better understanding of the mechanisms behind their control, and combining suitable elements for more sustainable and efficient control of pathogenic nematodes, at lower cost, with minimum impact on the environment and contributing to environmental services, holds promise, as is the case for agroforestry systems with coffee (Bertrand *et al.*, 2016).

RESISTANCE IN *C. CANEPHORA*: breeders have been assessing resistance against RKNs in *C. canephora* germplasm in relation to controlling these nematodes in *C. arabica* by grafting. More recently, selection of resistance to RKNs has also been implemented for *C. canephora* itself, especially for cv. Conilon in Brazil, against *M. incognita* and *M. paranaensis*. The widespread distribution and the aggressive nature of *M. incognita* in the west of São Paulo forced Brazilian researchers to rethink their options for efficient control measures other than chemicals. The introduction of *C. canephora* cv. 2258 from the CATIE germplasm collection, Turrialba, Costa Rica, provided strong resistance to *M. exigua* and resistance and/or tolerance to races 1, 2 and 3 of *M. incognita* (Fazuoli, 1986), as well as to *M. paranaensis* (Fazuoli *et al.*, 2002). The high level of resistance of cv. 2258, initially of 70%, was further improved through selection from fields highly infested with *M. incognita*. The resulting improved line, cv. Apoatã, is used as rootstock (Fazuoli *et al.*, 2002). *C. arabica* cv. Mundo Novo grafted on to *C. canephora* cv. Apoatã yielded 3.6 times greater than non-grafted plants grown

on fields infested with *M. incognita* race 1 (da Costa *et al.*, 1991). In Brazil, the planting of grafted coffee is now commonplace, even in non-infested areas. In some counties, especially in the west of São Paulo, grafted coffee has revived the coffee industry (Campos, 1997). Grafting on to cv. Apoatã rootstock has effectively become the only control method to enable sustainable cultivation of *C. arabica* in areas infested with *M. incognita* and/or *M. paranaensis* in Brazil (Fig. 15.8). Among numerous *C. canephora* clones of the coffee germplasm of CATIE in Turrialba, Costa Rica, just two clones (from one of which Apoatã originated) presented high levels of resistance to the main RKN species detected in Central America: *M. paranaensis*, *M. izalcoensis*, *M. arabicida* and *M. exigua* (Bertrand and Anthony, 2008). By multiplying these two clones *in vitro* and then crossing them in seed-producing parcels (crossed pollination), a new resistant rootstock cultivar, with a low segregation rate, was created and named cv. Nemaya (Bertrand and Anthony, 2008). Recently, the drought-tolerant *C. canephora* cv. Conilon clone 14 was identified with multiple resistance to *M. paranaensis* and to races 1 and 3 of *M. incognita* (Lima *et al.*, 2015). Since *C. canephora* is now widely affected by these aggressive RKNs, at least in Espírito Santo State, this clone can now be used as a rootstock for *C. canephora* (Barros *et al.*, 2011, 2014), as well as for *C. arabica*. In the western Amazon, Brazil, Santos *et al.* (2017a) identified 14 clones of *C. canephora* cv. BRS Ouro Preto, resistant to one population of *M. incognita* (Est I2). *C. canephora*

cv. Conilon Vitória Incaper 8142, Clone 6 V resistant to *M. paranaensis* performed well in an infested area of Minas Gerais, Brazil, standing out from susceptible materials (Fig. 15.9). Interestingly, late defence mechanisms, such as late hypersensitive-like reactions, have been observed in resistant *Coffea* germplasm, e.g. *C. canephora* cv. Conilon, clone 14 (Lima *et al.*, 2015), while late flavonoid accumulation in giant cells was observed without evidence of hypersensitive reaction in *C. canephora* cv. Nemaya and one *C. arabica* Ethiopian wild accession (Villain *et al.*, 2015).

Breeding for rootstocks has traditionally focused on *C. canephora*, *Coffea congensis* and *Coffea dewevrei* germplasm as a rich source of resistance to nematodes and other pathogens, due to their interesting vigour and abundant root systems (Gonçalves and Silvarolla, 2001). However, resistance genes found in wild or semi-wild lines of *C. arabica* from Ethiopia or Yemen could be used in coffee rootstock breeding programmes for implementing interspecific hybridizations with resistant diploid *Coffea* spp. lines. For example, the interspecific hybrid 'Arabusta' (*C. canephora* × *C. arabica*), in addition to nematode resistance, could provide better adaptability than *C. canephora* to the cooler coffee-growing areas at altitude, besides strong vigour (Capot, 1972; Berthaud, 1978a,b).

RESISTANCE IN *C. ARABICA*: resistance to *M. incognita*, *M. exigua* and *M. paranaensis* was found in many Brazilian coffee lines bearing the gene *Mex-1*; for example, *C. arabica* cv. IPR-100 with resistance to *M. incognita* race 1 (Kanayama *et al.*, 2009;



Fig. 15.8. Susceptible *Coffea arabica* cultivars non-grafted versus grafted on to *Coffea canephora* cv. Apoatã (IAC 2258) resistant rootstock in Brazil: (a) non-grafted cv. Catuaí infected by *Meloidogyne incognita* on left; (b) non-grafted cv. Obatã infected by *Meloidogyne paranaensis* on front row. (Photographs courtesy of S. Salgado.)

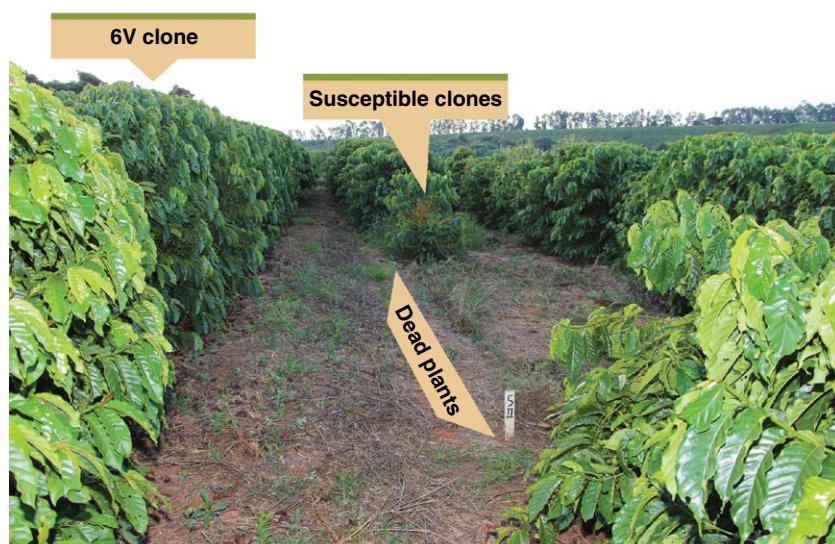


Fig. 15.9. *Coffea canephora* var. Conilon Vitoria INCAPER 8142 clone '6V' resistant to *Meloidogyne paranaensis* next to dead plants of a susceptible clone '5 II'. (Photograph courtesy of S. Salgado.)

Salgado *et al.*, 2014). Progenies from crosses between Catuai and Amphilobos cultivars also provided resistance against *M. paranaensis* and *M. incognita* (Perez and Carneiro, 2017, unpublished results) (Fig. 15.10). *C. arabica* genotype UFV 408-28 was resistant to *M. incognita* races 1, 2 and 3, with an average 87% reduction of *M. incognita* population densities compared to a susceptible control, with evidence of hypersensitive-like responses to these nematodes. In contrast, UFV 408-28 proved susceptible to *M. exigua* and *M. paranaensis* (Albuquerque *et al.*, 2010). This material, originated from crosses with cv. Amphilobos, continues to be improved to enhance its production potential in order to replace the *C. canephora* rootstock cv. Apoatã IAC 2258, or for direct planting.

Some progenies of *C. arabica* cv. Sarchimor (derived from Villa Sarchi $\times$ Timor Hybrid) and Icatu (advanced line derived from *C. arabica* $\times$ *C. canephora*) have shown moderate resistance to race 3 of *M. incognita*. Some Icatu progenies have shown resistance to *M. paranaensis* (Shigueoka *et al.*, 2016). Different levels of resistance to *M. exigua* have been observed on progenies derived from crossing *C. arabica* cv. Catuai and cv. Timor Hybrid (Rezende *et al.*, 2013) and also on Catimor cultivars (Pereira *et al.*, 2012). The Timor Hybrid, which is phenotypically an Arabica coffee, has frequently been used in *C. arabica* breeding

programmes as a source of resistance to some *Meloidogyne* spp. and to *H. vastatrix* (Bertrand and Anthony, 2008; Setotaw *et al.*, 2013). This is the case for Sarchimor cultivars such as Obatã (IAC 1669-20), Tupi (IAC 1669-33) and IAPAR-59 in Brazil (Ferraz, 2008; Fazuoli *et al.*, 2012) and Parainema and Marsellesa from Honduras and Nicaragua, respectively, but also for the Catimor cultivars Costa Rica 95 and Lempira from Honduras (Bertrand and Anthony, 2008). All the Sarchimor and Catimor cultivars are resistant to *M. exigua* and *H. vastatrix*. Resistance to *M. exigua* is controlled by a simply inherited major gene, designated the *Mex-1* locus, from *C. canephora*, with incomplete dominance (Noir *et al.*, 2003; Alpizar *et al.*, 2007). Hypersensitive-like reactions and induced and constitutive defence mechanisms have been reported in resistant *C. arabica* cultivars and *C. canephora* cv. Apoatã bearing the *Mex-1* gene (Anthony *et al.*, 2005; Silva *et al.*, 2013). *Mex-1* gene markers may be used in the selection of resistant coffee genotypes, such as microsatellite markers (SSR) that cluster genotypes according to their response to *M. exigua* infection, which provides the potential to select progenies resistant to this nematode in coffee breeding programmes (Pereira *et al.*, 2016).

Several semi-wild Ethiopian accessions of *C. arabica* from the Centro Agronómico Tropical



Fig. 15.10. Resistant and susceptible *Coffea arabica* genotypes under evaluation in a field infested by *Meloidogyne paranaensis* in Minas Gerais State, Brazil. (Photograph courtesy of S. Salgado.)

de Investigación y Enseñanza (CATIE) collection showed high levels of resistance to *M. paranaensis* populations from Guatemala and Brazil (Anzueto *et al.*, 2001; Boisseau *et al.*, 2009). Some of these wild Ethiopian accessions with resistance to *M. paranaensis* have been used to create F1 hybrids, in particular by crossing with Sarchimor lines resistant to *H. vastatrix* (van der Vossen *et al.*, 2015). However, seven of these F1 hybrids were phenotyped as highly susceptible to *M. paranaensis* under controlled inoculations (Villain *et al.*, 2015; Xalapa, Veracruz, Mexico, unpublished). Since these hybrids possess many useful characteristics, such as high yield, excellent quality, adaptation to shade, resistance to other diseases, these lines can be grafted on to resistant rootstocks such as cv. Nemaya for use in areas infested by *M. paranaensis*.

CROP ROTATION AND INTERCROPPING: de Moraes *et al.* (1977) studied rotations with cotton, soybean and maize in *M. exigua*-infested areas and concluded that coffee cultivation could be continued after a 1-year rotation with these crops. Almeida and Campos (1991a,b) also studied rotations with bean, soybean, sorghum and *Panicum maximum* in *M. exigua*-infested areas and similarly concluded that a 1-year rotation with

these crops was sufficient to enable a return to cropping susceptible coffee cultivars. Carneiro and Carneiro (1982) screened 29 crops for rotation in *M. incognita*-infested coffee fields and found that only *Arachis hypogaea* and *Ricinus communis* were immune, while *Mucuna pruriens* var. *utilis* (= *Stizolobium deeringianum*) and *Crotalaria spectabilis* showed resistance to this nematode. There was no penetration of J2 or galls or egg masses of *M. paranaensis* and *M. incognita* races 1, 2, 3 and 4 in *Arachis pintoi* roots following inoculation, and this could provide a potential rotation crop (Santiago *et al.*, 2001). In summary, a 1-year rotation in areas infested with *M. exigua* or *M. coffeicola* is possible between planting coffee cultivars susceptible to these species, but not with land infested with *M. incognita* or *M. paranaensis*. Rotation with non-hosts of *M. incognita* and *M. paranaensis* remains a useful strategy to reduce densities in infested areas before planting coffee grafted on Apoatã, since its resistance is not complete.

Intercropping velvet bean between rows of coffee and incorporating at the flowering stage protects coffee from cold wind and improves soil texture, organic matter and nutrients, reducing the damage by *M. incognita* and *M. paranaensis* in sandy soils depleted in organic matter (Fazuoli

et al., 2002). Replacing organic matter in depleted soils can be useful in delaying coffee eradication due to damage by nematodes.

BIOLOGICAL CONTROL: this is especially important where organic coffee prohibits chemical use and demands a higher market price. The nematode parasitic bacterium *Pasteuria penetrans* has advantages in being resistant to heat, drought and pesticides in the field (Campos *et al.*, 1998). *P. penetrans* was found in coffee fields in Colombia (Baeza-Aragón, 1978) and in Brazil (Sharma and Lordello, 1992). In Brazil, 21–65% of *M. exigua* J2 in coffee fields were naturally infected by *P. penetrans* throughout the year (Maximiniano *et al.*, 2001). In Cuba, isolates of *Metacordyceps chlamydosporia* (= *Verticillium chlamydosporium*) isolated from coffee plantations have potential as biological control agents for RKNs of coffee (Hidalgo-Díaz *et al.*, 2000). In Brazil, predators and egg parasitic fungi have also been isolated from coffee plantations (Naves and Campos, 1991; Ribeiro and Campos, 1993). The efficacy of *Arthrobotrys conoides*, *Arthrobotrys musiformes*, *Purpureocillium lilacinum* (= *Paecilomyces lilacinus*) and *M. chlamydosporia* for the control of *M. exigua* in coffee was determined by Campos and Campos (1997). Some strains of *Paecilomyces*, selected for their high level of efficiency in controlling *Globodera rostochiensis* on potato (Carrión *et al.*, 2014; Lima-Rivera *et al.*, 2016), have also shown promise against *M. paranaensis* on coffee (Carrión *et al.*, unpublished).

Methods of diagnosis

The presence of galls on young roots and/or the presence of swelling with or without cracking or corky symptoms on older roots are evidence of RKN infection. However, root symptoms provide only an indication of RKN infection and are not reliable for species-specific diagnosis, which requires extraction and identification (see Chapter 4, this volume).

MOLECULAR DIAGNOSIS: Species-specific SCAR molecular markers have been developed for most RKN species parasitizing coffee in Latin America: *M. exigua*, *M. incognita*, *M. paranaensis*, *M. enterobii*, *M. arenaria*, *M. arabicida* and *M. izalcoensis* (Randig *et al.*, 2002, 2004; Correa *et al.*, 2013). Moreover, its use in multiplex-PCR provides a

reliable tool for monitoring complex RKN species communities present on coffee plantations and for detecting species at low densities, due to the high sensitivity of this technique (Randig *et al.*, 2002, 2004; Carneiro *et al.*, 2005b; Correa *et al.*, 2013; López-Lima *et al.*, 2015). Other advantages of this technique are that RKN DNA can be extracted directly from roots and amplified without rearing each species individually and that it enables RKN detection using any life stage of the nematode, compared with electrophoresis analysis, which requires females. However, for species recently described, such as *M. lopezi*, or for species present only in Africa, species-specific markers have yet to be developed. Variable sites of 18S and 28S nuclear rDNA regions can be used to develop new species-specific SCAR markers for identification of *M. exigua* (Herrera *et al.*, 2011b). Recent exploration of mitochondrial coding genome analysis of tropical *Meloidogyne* species, however, has provided strong support for haplotype-based diagnostics, offering a seemingly durable *Meloidogyne* diagnostic technique (Pagan *et al.*, 2015; Janssen *et al.*, 2016). Meanwhile, it remains prudent to include both isoenzyme electrophoresis analysis and molecular markers to conduct RKN species identification from field surveys.

MORPHOLOGIC AND ISOENZYMATIC DIAGNOSIS: Perineal patterns are helpful for the identification of most *Meloidogyne* species, but do not differentiate *M. incognita* from *M. paranaensis*, which is possible by electrophoresis (Carneiro *et al.*, 2000; Carneiro and Cofcewicz, 2008). All esterase phenotypes of RKN detected on coffee, except the recently described *M. lopezi* (Humphrey-Pereira *et al.*, 2014), are available in Carneiro and Cofcewicz (2008) and Villain *et al.* (2013). The esterase phenotype S1 of *M. incognita* was reported by Oliveira *et al.* (2006) for the first time in Brazil on *C. arabica* in São Paulo. The use of more than one enzyme, such as Mdh, can be useful when distinguishing species with the same esterase phenotype, e.g. *M. exigua* and *Meloidogyne naasi* (Carneiro *et al.*, 2016). Electrophoresis also allows the detection of undescribed species present in any coffee root samples, which makes this diagnosis essential for any survey study. So, although esterase (EST) isozyme phenotypes are viewed as dated, they remain an essential tool for identifying *Meloidogyne* spp.

Meloidogyne africana*, *Meloidogyne arabicida*, *Meloidogyne arenaria*, *Meloidogyne coffeicola*, *Meloidogyne decalineata*, *Meloidogyne megadora*, *Meloidogyne hapla*, *Meloidogyne inornata*, *Meloidogyne izalcoensis*, *Meloidogyne javanica*, *Meloidogyne kikuyensis*, *Meloidogyne lopezi*, *Meloidogyne enterolobii* (= *Meloidogyne mayaguensis*), *Meloidogyne morrocciensis*, *Meloidogyne oteifai* and *Meloidogyne thamesi

Distribution

In this current edition, we have shifted *M. coffeicola* to the minor species category, since its distribution has become increasingly limited in Brazil over the past decade, where it was described by Lordello and Zamith (1960). This reduced distribution appears to be related to the fact that this species mostly affects older coffee trees (Carneiro and Cofcewicz, 2008). However, surprisingly, *M. coffeicola* was detected in Vietnam at one locality of the Western Highlands in combination with *M. exigua* from *C. canephora* (Trinh *et al.*, 2013). This also constitutes the first reliable record of these two species outside of Latin America. It would consequently be useful to establish whether or not this is due to introduced infected material.

Alternatively, we have removed *M. konaensis* from this list due to its tenuous status with coffee. Indeed, Sipes *et al.* (2005) observed three esterase phenotypes for *M. konaensis*, of which the fast-band Est F1 from coffee matches to the Est P1 observed for all Brazilian populations of *M. paranaensis* (Carneiro *et al.*, 2004). Furthermore, of four populations of *M. konaensis* detected from cabbage, papaya, noni and canapum plants in Brazil, none was able to reproduce on coffee (Monteiro *et al.*, 2016). Both *M. paranaensis* and *M. konaensis* are likely present in Hawaii, but only the former seems to parasitize coffee. Additional studies are therefore required in order to restore *M. konaensis* as a coffee RKN in Hawaii, to overcome any doubt.

Two newly described species of RKN from coffee have also been added to the list, *M. izalcoensis* n.sp., described from the region of the Izalco volcano, El Salvador (Carneiro *et al.*, 2005a) and *M. lopezi* n.sp., described from a southern region of Costa Rica and closely related to *M. arabicida*, *M. izalcoensis* and *M. paranaensis*

(Humphreys-Pereira *et al.*, 2014). *M. izalcoensis* is present over a range of altitudes (430–1100 m), covering most of the altitudinal range where *C. arabica* grows in Central America, although the species seems to have a relatively small area of distribution on coffee, limited to the region from where it was described (Villain *et al.*, 2013). However, it has since been detected on coffee in Kenya and Tanzania, in addition to other crops in West Africa (Jorge-Junior *et al.*, 2016). *M. lopezi* causes very small galls on the fine roots, each containing a single female, with the egg mass exposed outside the gall tissues.

M. arabicida, also described from Costa Rica (López and Salazar, 1989), appears to have a distribution restricted to this country only (Villain *et al.*, 2013).

M. hapla has been detected in a few coffee plantations through Latin America and Africa, mostly at the higher altitudes of coffee-growing regions, in accordance with its general distribution in temperate climate regions (Villain *et al.*, 2013). Whitehead (1969a) and Bridge (1984) suggest that coffee is not a good host for this species, or for *M. javanica*.

M. arenaria has been recovered from coffee in Jamaica, El Salvador and Guatemala (Campos and Villain, 2005; Villain *et al.*, 2013). However, Carneiro *et al.* (2008) suggested that the taxonomic entities currently recognized as '*M. arenaria*' should be reviewed. Indeed, *Meloidogyne morrocciensis* was detected on coffee in El Salvador but with an esterase phenotype Est A3, previously considered as belonging to *M. arenaria* (Villain *et al.*, 2013) but which should, together with a Mdh N1 phenotype, be attributed to *M. morrocciensis* (Carneiro *et al.*, 2008).

M. enterolobii was first detected on coffee in Cuba (Sampedro *et al.*, 1989) and then in Guatemala and Costa Rica (Hernández *et al.*, 2004; Villain *et al.*, 2013). This species is widespread in the tropics and subtropics on various plant hosts, and is now considered as a major emerging RKN (Castagnone-Sereno, 2012). On coffee, its limited occurrence in low densities would indicate that coffee is not a suitable host for this species (Villain *et al.*, 2013), as observed in greenhouse experiments (Muniz *et al.*, 2009). To further support this, *M. enterolobii* was recovered from banana plants in a coffee plantation in Mexico, but attempts to reproduce this population on *C. arabica* failed (Villain *et al.*, 2015, unpublished).

M. inornata, described by Lordello (1956) from Brazil and redescribed by Carneiro *et al.* (2008), also occurs on coffee in Guatemala (Schieber and Sosa, 1960).

In Africa, where coffee is a major cash crop in a number of countries, there has been surprisingly little nematology activity on coffee, with only limited surveys providing a scant impression of the nematode distribution on coffee across coffee-producing countries. From the available data, *M. africana*, *M. decalineata*, *M. kikuyensis* and *M. megadora* appear to have quite restricted distributions. In Tanzania and the Democratic Republic of the Congo (DRC), where more data are available, numerous species of *Meloidogyne* occur on coffee (Campos and Villain, 2005). *M. decalineata* seemed to be common in Kilimanjaro and the Usambara Mountains of northern Tanzania (Swai, 1981). *M. kikuyensis* was also present in Kilimanjaro (Swai, 1981). *M. africana* occurred across Kenya and the DRC (Whitehead, 1959; Lordello, 1972). Bridge (1984) reported *M. decalineata* and other species of *Meloidogyne* across Tanzania. *M. megadora* is recorded from coffee (*C. arabica*, *C. canephora*, *C. congensis* and *C. eugenioides*) in Angola and Uganda (Whitehead, 1968, 1969a) and São Tomé, Democratic Republic of São Tomé and Príncipe (Maleita *et al.*, 2016). *Meloidogyne* sp. has also been found on coffee in Zimbabwe (Way, 1981). *M. oteifai* occurs in Zaire (Elmiligy, 1968). However, it would appear there has been some confusion over the identity of some species in Africa. With progress in molecular techniques, in particular the use of mitochondrial coding genome analysis, *M. decalineata* and *M. oteifai* have since been synonymized with *M. africana*, based on their Cox1 sequence (Janssen *et al.*, 2017). From a limited number of field samples in Kenya, Tanzania and Uganda, the occurrence of several species, such as *M. africana*, *M. hapla*, *M. incognita*, *M. izalcoensis* and *M. javanica*, were variously present, including the occurrence of at least one undescribed species, indicating a potentially diverse and expanding diversity of *Meloidogyne* species on coffee in East Africa, its centre of origin (Janssen *et al.*, 2016, 2017; Jorge-Junior *et al.*, 2016; D.L. Coyne, Nairobi, 2017, personal communication).

M. thamesi has been found in coffee soil in India (Kumar, 1984).

Additionally, two isolates of RKN collected on coffee from El Salvador displayed unknown

esterase phenotypes ('M1F1a' and 'Sa4') and should be studied morphologically and molecularly to confirm their identity.

Symptoms of damage

The outbreak of *M. coffeicola* in Paraná State, Brazil, resulted in the death of numerous coffee trees and had a major economic impact (Lordello and Zamith, 1960). *M. coffeicola* causes peeling and cracking of roots, but does not produce galls. Females are found in older root tissue, especially on the taproot, and egg masses are predominantly external, emerging from cracks. *M. arabicida* causes corky root symptoms, similar to those caused by *M. paranaensis*, with numerous swellings evolving into extensive developments of corky tissues on the taproot up to the collar, and on the primary and secondary roots. Considerable cracking of the cortical tissues is also observed (López and Salazar, 1989; Bertrand *et al.*, 2000). These symptoms are very similar to those observed on coffee trees infected by *M. paranaensis* in Brazil, Guatemala or Mexico. Both *M. paranaensis* and *M. arabicida* female development leads to peridermal disruption with exterior egg masses (Bertrand *et al.*, 2000). In the field, infected plants show a progressive decline with leaf chlorosis and leaf fall, followed by flower and fruit fall, leading to plant death within 2–4 years after planting. Symptoms caused by *M. izalcoensis* include swellings on non-lignified, white secondary roots and also on woody roots, including the taproot, which may become slightly corky and necrotic (Villain *et al.*, 2013). *M. arenaria* causes large root swellings without corky formation across the whole root system, including the taproot up to the collar, and with dramatic induction of adventitious roots. Similar symptoms have been observed with *M. morocciensis* on *C. arabica* in Guatemala (Villain, unpublished). On the very few infected *C. arabica* trees observed in Guatemala and Costa Rica, large galls (larger than those produced by *M. exigua*) and some swellings without cortex cracking have been observed (Villain *et al.*, 2013). Converseley, Rodríguez *et al.* (1995) reported severe symptoms by *M. morocciensis* in Cuba, similar to those caused by *M. paranaensis* or *M. arabicida*: corky roots, with cortex cracking up to the collar. *M. hapla* causes light root galling and swellings, different to the symptoms of other

species occurring in Tanzania (Bridge, 1984). In Brazil, it causes typical galls of various size, but similar to those caused by *M. exigua*. Necrosis and induction of lateral roots also occur close to the galls (Lordello, 1982); similar symptoms are observed on *C. arabica* in El Salvador (Villain *et al.*, 2008). *M. africana* usually cause small to moderate-sized galls from 1 to 5 mm in diameter, mainly at the root tips, from where numerous rootlets are produced in reaction (Fig. 15.11). It is reported causing poor growth on both *C. arabica* and *C. canephora* on nursery seedlings, as well as affecting mature coffee trees (Campos and Villain, 2005).

Biology and life cycle

The biology and life cycle of most of these minor RKN species are still unknown. *M. arenaria*, *M. javanica* and *M. enterolobii* reproduce by mitotic parthenogenesis, while *M. hapla* reproduces by meiotic parthenogenesis or by amphimixis (Castagnone-Sereno *et al.*, 2013).

Other hosts

M. coffeicola has been found only on *Praxelis diffusa* (= *Eupatorium pauciflorum*) and *Psychotria leiocarpa* (= *Psychotria nitidula*) (Lordello and Lordello, 1972; Jaehn *et al.*, 1980), hence Lordello and Zamith (1960) hypothesized that this species became a pathogen of coffee after the clearing of forests where it was a native species. *M. arenaria*, *M. hapla* and *M. javanica* infect a



Fig. 15.11. Small, rounded galls caused by *Meloidogyne africana* on *Coffea arabica* roots in Tanzania; note that galls are mainly localized at root tips. (Photograph courtesy of D. Coyne.)

great number of crops and weeds in many countries. The Est A1 phenotype of *M. arenaria* was also observed on a sample of *Impatiens* sp., a common weed in Central American coffee plantations (Villain *et al.*, 2013). In Africa, *M. africana* is recorded infecting maize, cowpea, clove, potato, pyrethrum and (as *M. megadora*) numerous coffee species, and *M. kikuyensis* from cowpea (Whitehead, 1969a). In Brazil, *M. thamesi* infects cocoa, *Turnera ulmifolia*, *Spondias mombin* (= *Spondias lutea*), *Rivina humilis*, *Petiveria alliacea* var. *tetrandra* (= *Petiveria hexaglochin*) and *L. sibiricus* (Ponte, 1977; Lordello, 1984), while *M. inornata* infects soybean (Ponte, 1977). *M. enterolobii* is reported worldwide on many crops and is considered a major threat for many crops (Castagnone-Sereno, 2012). *M. izalcoensis* was recovered from cabbage in Benin and from tomato and sweet pepper in Tanzania (Jorge-Junior *et al.*, 2016). *M. moroccensis* was described from peach rootstock in Morocco (Rammah and Hirschmann, 1990) and also affects soybean in Brazil (Mattos *et al.*, 2016).

Disease complexes

Under controlled conditions, only the inoculation of both *M. arabicida* and *F. oxysporum* resulted in corky root symptoms on *C. arabica* cvs. Caturra and Catuai, while *M. arabicida* alone caused gall formation only, without corky root symptoms. In fields planted with cultivars susceptible and resistant to *M. arabicida*, only the susceptible developed the corky root symptoms. The predisposition of the host to *M. arabicida* consequently plays a major role in this complex aetiology (Bertrand *et al.*, 2000; Rivillas *et al.*, 2015).

In the case of *M. arabicida*, *M. paranaensis*, *M. incognita* or *M. coffeicola* parasitism (Anzueto, 1993, and Bertrand *et al.*, 2000), the development of females close to the surface of the roots, and rupturing of the cortex, which leads to external emergence of egg masses (unlike with *M. exigua*), likely favours infection by secondary pathogens, such as *Fusarium*, leading to cracking and corky root symptoms.

Economic importance

Although there is no information available in Tanzania on the actual yield losses caused by nematodes, yield losses of trees severely infected

with the African coffee root knot nematodes were estimated by Bridge (1984) to be in the region of 20% under optimum conditions, extending to the point of non-productivity. Nematode damage will also cause premature fruit drop, twig dieback and defoliation, nutrient deficiency and stunted growth. Although *M. arabicida* distribution is restricted to a small area in Costa Rica and has yet to be detected elsewhere, this parasite presents a threat to coffee-growing regions; where present, its economic impact can be so severe it results in the abandonment of coffee plantations (Anon., 1989).

Management measures

The control measures described for *M. exigua*, *M. incognita*, *M. coffeicola* and *M. paranaensis* are likely to be effective for the control of African RKNs, but the application of these measures on a practical basis in African countries is uncertain. However, the assessment of *Coffea* species, crosses and selections against RKNs in Tanzania by Bridge (1984) indicated some resistance, and that grafting on to resistant rootstocks could be useful. Reports from the Kenya Coffee Research Station, cited by Whitehead (1968), also indicate some resistance to *Meloidogyne* sp. in *Coffea carrisoi*, Conuga or Congusta coffee (hybrid of *C. congensis* and *C. canephora*) and some lines of *C. congensis* in Angola. Whitehead (1969b) regarded coffee as highly resistant to both *M. javanica* and *M. kikuyensis*.

Bertrand *et al.* (2002) found that resistance to the corky root disease complex, caused by *M. arabicida* and *F. oxysporum*, on *C. arabica* in Costa Rica was heritable and that genetic resistance to *M. arabicida* was an effective strategy against the complex. By using *C. canephora* rootstocks, it was possible to reduce mortality substantially in the field and to reduce by half the number of plants with corky root symptoms. *C. canephora* rootstock cv. Nemaya is resistant to *M. arabicida* and *M. izalcoensis* (Bertrand and Anthony, 2008). There has been no assessment of coffee resistance against *M. lopezi*, due to its recent description.

Pratylenchus

Root lesion nematodes (RLNs) *Pratylenchus* spp. are considered major parasites of Arabica and

Robusta coffee in many coffee-producing countries or regions (Villain, 2008; Wiryadiputra and Tran, 2008; Rivillas *et al.*, 2015). Species known to occur on coffee include *Pratylenchus brachyurus*, *P. coffeae*, *Pratylenchus delattrei*, *Pratylenchus good-eyi*, *Pratylenchus gutierrezii*, *Pratylenchus loosi*, *Pratylenchus neglectus*, *Pratylenchus panamaensis*, *Pratylenchus penetrans*, *Pratylenchus pratensis*, *Pratylenchus vulnus* and *Pratylenchus zaei*. Among these species, *P. brachyurus* and *P. coffeae* are the most widespread in the tropics (Inomoto and Oliveira, 2008). However, in São Paulo, Brazil, *P. brachyurus* is more widely distributed, while *P. coffeae* occurs in higher densities, causing greater damage where it occurs (Kubo *et al.*, 2004).

Distribution

P. coffeae, described from coffee from Java Island (Zimmermann, 1898; Sher and Allen, 1953), is the most widespread and damaging RLN on coffee worldwide (Campos and Villain, 2005; Handoo *et al.*, 2008; Inomoto and Oliveira, 2008). In some countries, it has long been considered a major threat for *C. arabica*, such as in Guatemala and El Salvador (Villain *et al.*, 2008) or in India (Dhanam and Sreedharan, 2008), but also for *C. canephora*, especially in Indonesia and Vietnam (Wiryadiputra and Tran, 2008). Indeed, in Vietnam, *P. coffeae* is the dominant plant parasitic nematode species on both Robusta and Arabica coffee, occurring in all coffee-growing areas (Tran, 2002). *P. coffeae*, which has a large pantropic distribution, may have the same geographical origin as banana and plantain, that is, the Pacific islands and neighbouring Asian countries, from which it may have been spread through the transfer of infected plant material (Bridge *et al.*, 1997).

P. brachyurus is present in many coffee-growing regions in Brazil and appears to be the most widely distributed RLN in this country, especially in São Paulo and Minas Gerais (Inomoto and Oliveira, 2008). It was also reported on coffee in Vietnam (Nguyen and Nguyen, 2001). *P. pratensis* has been reported from one locality in south India by Somasekhar, cited by Whitehead (1969b), and *P. loosi* from Sri Lanka by Hutchinson, cited by Whitehead (1968). *P. goodeyi* occurs frequently on coffee in Tanzania (Bridge, 1984). *P. vulnus* was detected on coffee in Minas Gerais State, Brazil (Ferraz,

1980; Monteiro *et al.*, 2001). *P. zae* has been found only in soil samples associated with graminaceous weeds in coffee plantations, so its status as a coffee parasite is uncertain (Inomoto and Oliveira, 2008).

Two species morphologically similar to *P. coffeae* were described from coffee in Panama and Costa Rica, *P. panamaensis* (Siddiqi *et al.*, 1991) and *P. gutierrezii* (Golden *et al.*, 1992), respectively. Siddiqi (2000), however, considered *P. gutierrezii* as a junior synonym of *P. panamaensis*, using morphometrics, while molecular characterization revalidated *P. gutierrezii* as a separate species (Zamora-Araya *et al.*, 2016). In Vietnam, *P. delattrei*, *P. neglectus* and *P. penetrans* were additionally reported from coffee (Nguyen and Nguyen, 2001).

Due to difficulties in differentiating *Pratylenchus* spp. morphologically, many populations collected from coffee from many countries remain unidentified, as is the case across Central America and Brazil (Campos and Villain, 2005). This is especially the case for the complex of amphimictic species morphologically similar to *P. coffeae*, such as *P. gutierrezii*, *P. panamaensis*, *Pratylenchus pseudocoffeae* (not yet recorded from coffee) and likely undescribed species (Campos and Villain, 2005; Zamora-Araya *et al.*, 2016). Additional studies are consequently necessary to determine the diversity of *Pratylenchus* spp. parasitizing coffee through an integrated taxonomy approach, involving molecular tools and pathogenicity assessment of the detected taxa.

Symptoms of damage

The same atypical aerial symptoms mentioned for RKN infections are also observed for coffee RLN infections: stunting, chlorosis, leaf fall, progressive decay with intensity varying according to the nematode species, the *Coffea* species, cultivar and age, and the agroecological conditions.

Unlike RKNs, however, RLNs provoke root symptoms, which are not always easy to recognize, with atypical necrosis observed mainly on fine roots, due to RLNs feeding on the cortical parenchyma cells. With the more aggressive RLN species, this necrotic process of absorbing fine roots leads to rotting and destruction of the entire root system and death of infected plants. Due to the unspecific aerial and root symptoms,

the occurrence of RLNs likely remains regularly unnoticed, with soil fertility issues or other soil-borne diseases blamed (Villain *et al.*, 2002; Villain, 2008).

In Guatemala, an RLN population, likely of *P. panamaensis* according to Zamora-Araya *et al.* (2016), proved highly pathogenic on *C. arabica* under controlled conditions (Fig. 15.12) and in the field (Villain, 2000; Villain *et al.*, 2002), affecting both the quantity and quality of coffee produced (Fig. 15.13). The size and yield of coffee beans were strongly correlated negatively with RLN root densities (Villain, 2000; Villain *et al.*, 2008), with average yields of 6 t/ha in the least infested plots down to 0.5 t/ha in the most infested plots. Bean size followed a similar trend, while high rates of plant death were also observed in the most infested plots, with up to 76% mortality 4 years after planting, with densities of 125 nematodes/g root. Consequently, particular attention is required for this species, which is highly pathogenic and likely has a wide distribution.

In Brazil, *P. coffeae* populations collected from coffee caused root destruction of *C. arabica* seedlings following reinoculation (Kubo *et al.*, 2002; Inomoto *et al.*, 2007). In the north-east of Brazil, a severe infestation of *P. coffeae* was reported in *C. arabica* fields previously cultivated with yam (*Dioscorea cayennensis*) and had been abandoned due to *P. coffeae* (Moura *et al.*, 2003). Severe root necrosis in the cortex, and even in the collar area of the stem, was observed, leading to severe decay of trees and death in up to 70% of the plantation. *P. coffeae* is the most destructive nematode on *C. arabica* in south India (Palanichamy, 1973). In Indonesia, *P. coffeae* is reported as highly destructive, causing production losses between 29 and 79% on *C. canephora* cv. Robusta plantations (Toruan-Mathius *et al.*, 1995). In Vietnam (Fig. 15.14), *P. coffeae* is the only RLN species reported from Robusta coffee in the Western Highlands (Tran, 2002), where it is considered among the main causes of the leaf yellowing disease (Trinh *et al.*, 2009a; Le *et al.*, 2013; Vu *et al.*, 2014).

P. brachyurus reduces plant and root growth, and results in shedding of leaves and root darkening (Kubo *et al.*, 2004). Infection by *P. goodeyi*, *P. loosi*, *P. pratensis* and *P. zae* on coffee is not clearly defined. *P. brachyurus* and *P. zae* have been reported from the rhizosphere



Fig. 15.12. Eight-month-old *Coffea arabica* seedlings, six months after inoculation of *Pratylenchus panamaensis* from Guatemala, at different inoculum levels. From left to right: 0 (control), 100, 200 and 400 inoculated individuals. (Photograph courtesy of L. Villain.)



Fig. 15.13. Infestation 'hotspot' of *Pratylenchus panamaensis* in a plantation of *Coffea arabica* cv. Caturra in Guatemala during the dry season, showing a dramatic increase of hydric stress. (Photograph courtesy of L. Villain.)



Fig. 15.14. Young plantation of *Coffea canephora* infected with *Pratylenchus coffeae* in the Western Highlands of Vietnam, showing symptoms of chlorosis, plant stunting and death. (Photograph courtesy of P.Q. Trinh.)

of *C. arabica* fields previously planted with sugarcane in Hawaii, but both species tended to disappear 3 years after planting, indicating that coffee is a poor host (Schenk and Schmitt, 1992).

Biology and life cycle

P. coffeae is a bisexual species that reproduces by obligatory amphimixis, so males are frequent, as they are for *P. panamaensis*, *P. gutierrezi*, *P. pratensis*, *P. loosi* and *P. goodeyi*. However, *P. brachyurus* and *P. zae* are monosexual species (males absent or rare) that reproduce by mitotic parthenogenesis (Roman and Triantaphyllou, 1969). *Pratylenchus* spp. lay their eggs in the root cortical tissue, as well as on the surface of roots. All stages feed on cortical tissue, and extended periods of feeding result in cell death and necrotic lesions, hence their name (Moens and Perry, 2009). *P. coffeae* eggs hatch in 6–8 days at 28–30°C; the first appearance of adults is observed 15 days

after hatching at 25–30°C on *Solanum tuberosum* tubers (Siddiqi, 1972). Under these conditions, the average life cycle duration is 27 days. For *P. brachyurus*, under optimum temperature conditions (30 or 35°C), one cycle takes 4 weeks on maize, while at 10°C the cycle remains uncompleted after 14 weeks (Siddiqi, 1976a). *P. zae* completes its cycle within 35–40 days (Siddiqi, 1976b). *P. loosi*, which is more adapted to cooler climates than the species previously mentioned, completes its life cycle in 45–48 days, comprising 15–17 days for the eggs to hatch, 15–16 days as juveniles and 15 days as adults before egg laying (Siddiqi, 1977). More information on the population dynamics of RLNs that infect coffee are provided by Inomoto and Oliveira (2008).

Races

Cross-inoculation studies on *C. arabica* with *P. coffeae* populations recovered from seven different hosts revealed differences in reproduction and

pathogenicity, indicating a physiological specialization in this species (Kumar and Viswanathan, 1972). Differences in aggressiveness among *P. coffeae* populations have been reported in Brazil (Silva *et al.*, 2001). However, since species-level identifications have proved difficult for most RLNs detected from coffee, the information available on intraspecies-specific diversity, in terms of pathogenicity or biology, needs to be treated with caution.

Survival and means of dissemination

In the absence of hosts, *P. coffeae* can survive for 8 months in moist soil (Siddiqi, 1972). Studies with *P. brachyurus* populations from different crops, however, show that they are influenced strongly by available soil moisture (Siddiqi, 1976a). Both species are polyphagous and can multiply on a diversity of grasses, especially *P. brachyurus*, and so coffee plantations established after pastures may be highly infested. A wide range of weeds and cover crops that are sometimes used between coffee rows are likely responsible for maintaining high levels of RLNs in coffee (Kubo *et al.*, 2015). *P. coffeae* has the capacity for anhydrobiosis, enhancing its tolerance to adverse environmental conditions such as low soil moisture, and is able to survive for 9 months using this mechanism (Tsai, 2008). The main means of dissemination of RLNs are similar as for RKNs, described above.

Environmental factors affecting parasitism

Sandy soils are more favourable than clay soils for horizontal migration of *P. brachyurus* and *P. zeae* (Siddiqi, 1976b). A light soil texture, with soil particles around 2 mm, was favourable to nematode movement, compared with particle sizes of 0.05–1 mm (Dhanam and Sreedharan, 2008).

In Guatemala, the population dynamics of *Pratylenchus* sp. were studied over 3 years at two altitudes (450 and 1200 m). At both sites, two population peaks were observed, one during the dry season and the other at the beginning of the rainy season (Campos and Villain, 2005). Population fluctuations were not directly related to rainfall pattern, but rather to the coffee tree phenological cycle. The two annual population peaks coincided with coffee root-growing peaks,

while the lowest population densities occurred during coffee berry maturation. This may be linked with certain characteristics of coffee tree physiology, with the beans working like physiological sinks during maturation, causing an important change in carbon allocation at the expense of the roots, especially the secondary roots that stop growing and even die (Cannell and Huxley, 1969; Cannell, 1971). Another interesting aspect of *Pratylenchus* population dynamics is that densities fluctuate hugely, over very short periods, demonstrating their high pathogenic capacity on coffee.

Soil temperature is an important factor in the development of *Pratylenchus* spp. *P. coffeae* seems to have its temperature optimum at around 30°C for multiplication, while its parasitic capacity is nearly halted at 35°C, from observations on *Citrus taitensis* (= *Citrus jambhiri*) and *Glycine max* (Radewald *et al.*, 1971; Acosta and Malek, 1978). In contrast, *P. loosi* is less thermophilic and, although this varies with host, its temperature optimum appears to be around 20°C (Sivapalan and Gnanapragasam, 1975; Siddiqi, 1977; Gnanapragasam, 1982). Among *Pratylenchus* spp. populations collected in Guatemala, two populations morphologically similar to *P. coffeae* (one highly and the other slightly pathogenic on *C. arabica*) had the same temperature optimum for reproduction, between 27 and 29°C, on carrot discs *in vitro*, while reproduction decreased severely when temperatures fell to 24°C or raised to 30°C. In contrast, two populations, one from Guatemala and one from El Salvador, that are both considered as the same species (Hervé, 1997), likely *P. panamaensis* according to Zamora-Araya *et al.* (2016), showed a temperature optimum for multiplication between 24 and 27°C (Hervé, 1997; Villain, 2000). In Nicaragua, the highest *Pratylenchus* spp. densities in coffee plantations were observed where nitrogen-fixing shade species were present (Herrera *et al.*, 2011a).

Avelino *et al.* (2009) observed in Costa Rica that high-density planting of coffee, with within-row distances less than 0.9 m, favoured high *P. coffeae* densities, as well as of *M. exigua*. Interspecies competition between *P. coffeae* and *M. paranaensis* was observed in Guatemala and between *P. coffeae* and *M. exigua* in Costa Rica (Hervé *et al.*, 2005). Competition between *P. coffeae*

and *M. exigua* has also been observed by Avelino *et al.* (2009) in Costa Rica.

Other hosts

P. brachyurus infects a wide diversity of tropical and subtropical crops (see various relevant chapters in this volume). Grasses, which commonly occur within coffee rows in Brazil due to the larger within-row distances, such as *Melinis minutiflora*, *Hyparrhenia rufa* and *Brachiaria* spp., are good hosts for *P. brachyurus* (Lordello, 1972; Kubo *et al.*, 2004). *P. coffeae* has a wide host range (Nickle, 1984), as does *P. zae* (Tenente *et al.*, 2002). *P. coffeae* represents a major pest of other crops, such as *Musa* spp. (see Chapter 17, this volume), *Citrus* spp. (see Chapter 12, this volume), *Dioscorea* spp. (see Chapter 8, this volume), *Ipomoea batatas* and *S. tuberosum* (see Chapter 7, this volume). This species is also present on many weeds (Loof, 1991). *P. goodeyi* is an important parasite of banana in the highlands of East Africa (see Chapter 17, this volume). *P. loosi* is an important parasite of tea (*Camellia sinensis*) in many regions of Asia (Whitehead, 1969b; see Chapter 16, this volume).

Management measures

As for RKN control, the most important measure to control RLNs is prophylactic action to prevent their dissemination by nursery seedlings or soil (see above). Effective control of the highly destructive *P. coffeae* requires the integration of several options, such as regulatory, physical, cultural, chemical, biological and plant resistance (Villain, 2008). Control of *P. coffeae* by chemical or physical means is not often economical or practical, because of endoparasitism. Planting in suspected infested areas should be conducted with Robusta (*C. canephora*) or Robusta-Arabica grafted plants (Dhanam *et al.*, 2011).

The application of carbosulfan (25 EC Marshal™) applied as a soil drench in Arabica coffee fields, in January, April and July, significantly reduced *P. coffeae* densities until March of the following year, increasing coffee growth and the development of new roots (Dhanam and Sreedharan, 2008).

In the field, treatment with rhizobacteria to manage *P. coffeae* increased the activity of

peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL), reduced *P. coffeae* and improved coffee yields (Senthikumar and Deivanami, 2016).

Early studies on the efficacy of synthetic nematicides, oxamyl, phenamiphos and aldicarb against *P. coffeae* in coffee nurseries in El Salvador indicated that increased coffee yields were obtained in the second year (Abrego, 1974). Good control of *P. coffeae* was also obtained with phenamiphos, which remained effective under field conditions for 90 days after application (Kumar, 1982). On the other hand, Villain *et al.* (2000) observed that terbufos applications (1–2 g/plant) suppressed *Pratylenchus* sp. (likely *P. panamaensis*) in coffee roots only during the first 2 years after planting. This resulted in a significant decrease of plant mortality in ungrafted *C. arabica* plots, but not in a significant increase in yield.

In a search for resistance genes in *C. arabica*, progeny from Ethiopia and Yemen, the two most important centres of *C. arabica* genetic diversity, were highly susceptible to *Pratylenchus* sp. from Guatemala (Villain *et al.*, 2004).

C. canephora genotypes, studied by Dhanam and Sreedharan (2008), were considered the most resistant to *P. coffeae*, followed by *C. liberica*, while *C. arabica* genotypes were considered highly susceptible. Variable resistance to *Pratylenchus* populations from Guatemala was observed among *C. canephora* progenies (Villain *et al.*, 2004), in accordance with the substantial genetic variability observed within this species (Cubry *et al.*, 2008; Musoli *et al.*, 2009). The two reciprocal crossings of the two parent clones of the rootstock *C. canephora* cv. Nemaya, which is also resistant to *Meloidogyne* spp. from Central America (see above), were the most resistant progenies to *Pratylenchus* sp.; pre- and post-infective resistance factors were observed on *C. canephora* cv. Nemaya (Villain, 2000; Villain *et al.*, 2004). At the pre-infective stage, early penetration studies showed that *C. canephora* cv. Nemaya was unattractive to *Pratylenchus* spp., compared with the high attractiveness of *C. arabica* cv. Catuai. No histological structure likely to prevent or hinder penetration by nematodes was detected. At the post-infective stage, the poor reproduction of *Pratylenchus* spp. observed on *C. canephora* may be related to the constitutive presence of many polyphenols, especially

in cv. Nemaya roots. Indeed, a strong correlation between resistance to *P. coffeae* with the total polyphenol content was observed in roots among numerous *C. canephora* clones in Indonesia (Toruan-Mathius *et al.*, 1995). In addition, cambium lignifications, close to nematode lesions in the cortical parenchyma, were observed on cv. Nemaya roots, indicating that inducible resistance mechanisms were also occurring (Villain *et al.*, 2004). If the main *Pratylenchus* resistance factors are linked to the phenolic metabolism, it is to be hoped that this resistance is not very specific (Dalmasso *et al.*, 1992) and would thus provide the plant with an acceptable level of resistance to *Pratylenchus* species and/or pathotypes. In Brazil, *C. canephora* cv. Conilon is resistant and *C. canephora* cv. Robusta is susceptible to *P. coffeae* strain K5 (Tomazini *et al.*, 2003). In India, *C. canephora* cv. Robusta is more tolerant to *P. coffeae* than *C. arabica* or *C. liberica* var. *dewevrei* (= *Coffea excelsa*) (Anon., 1974). The use of *C. canephora* cv. Robusta as a rootstock therefore offers the most promising means of control (Palanichamy, 1973). In fact, Schieber and Grullon (1969) also suggested the use of *C. canephora* var. Robusta in Guatemala as a source of resistance for rootstocks in grafted plants. Since grafting on

to *C. canephora* cv. Robusta at the cotyledon stage was perfected by Reyna (1968), this practice has become increasingly common for controlling nematodes in Guatemala. Grafting on common *C. canephora* rootstock provides efficient control of *Pratylenchus* spp. (Fig. 15.15), keeping densities at very low levels, even without chemical control (Villain *et al.*, 2000, 2004). Grafting on to *C. canephora* also has the advantage of maintaining both physical and chemical bean qualities, as well as the organoleptic beverage quality of *C. arabica* (Villain *et al.*, 2001). Grafting was achieved successfully for varieties susceptible to *P. coffeae* much earlier on to Conuga hybrid (*C. congensis* × *C. canephora* var. *ugandaea*) and on to *C. canephora* cv. Robusta in India (Siddiqi, 1972).

Consequently, and similar for RKN control, prophylactic measures, the currently available sources of resistance to RLNs, especially for use as rootstock for Arabica coffee, together with the increasing and recent knowledge on the use of beneficial microorganisms to control plant parasitic nematodes, allow for an environmentally friendly and efficient integrated management of phytopathogenic nematodes without relying on conventional chemical control in coffee plantations.

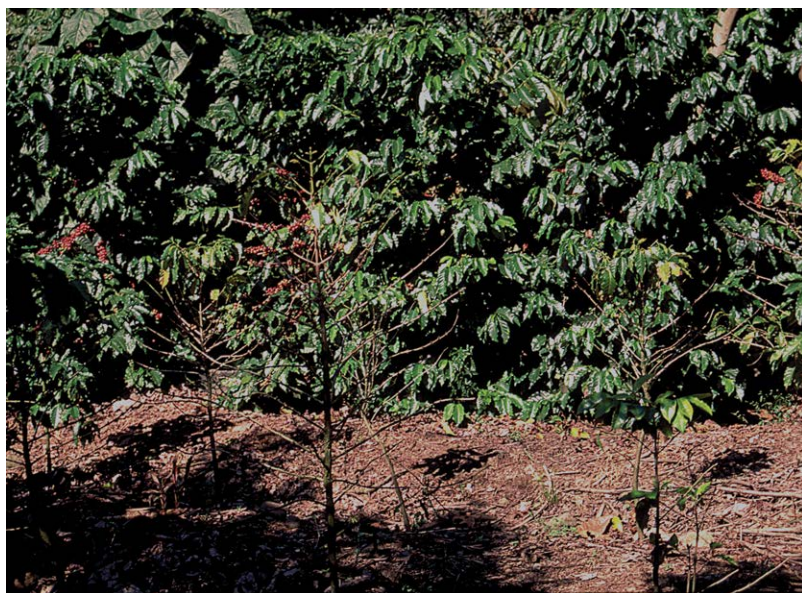


Fig. 15.15. Experimental field plot of 3-year *Coffea arabica* cv. Caturra infected with *Pratylenchus panamaensis* in Guatemala: defoliated and near-dead, non-grafted plants in the foreground versus highly productive and well-foliated plants grafted on to *Coffea canephora*. (Photograph courtesy of L. Villain.)

Other nematode parasites of coffee

Radopholus similis was reported from coffee in Java by Zimmermann (1898). This nematode was considered the most harmful nematode in that country, and second only in importance to *P. coffeae* (Campos and Villain, 2005). Three *Radopholus* species have since been recovered from coffee in Vietnam, including two species described from coffee. *Radopholus duriophilus*, first described from durian, *Durio* spp. (Nguyen *et al.*, 2003), was later found on Robusta coffee in Krong Ana District (Trinh *et al.*, 2004). *Radopholus arabocoffeae* is described from *C. arabica* in Krong Nang (Trinh *et al.*, 2004) and occurs widely on Arabica coffee in the provinces of Gia Lai, Daklak, Dak Nong and Lam Dong (Trinh *et al.*, 2009a, 2012; Le *et al.*, 2013; Vu *et al.*, 2014). In the field, no correlation was determined between *R. arabocoffeae* density and yellow leaf symptom (Le *et al.*, 2013; Vu *et al.*, 2014). However, dieback and deciduous symptoms on Arabica coffee were clearly observed in Daklak Province when *R. arabocoffeae* densities were high (Trinh *et al.*, 2009a). Moreover, Trinh *et al.* (2011) observed

that *P. coffeae* mainly destroyed lateral roots rather than taproots, whereas *R. arabocoffeae* reduced taproot length rather than affecting the lateral roots (Fig. 15.16). *Radopholus daklakensis* was described from Robusta coffee in Daklak Province (Trinh *et al.*, 2012). *Radopholus* was absent in the coffee-growing areas from the north and centre of Vietnam.

Among other species of nematodes parasitic to coffee, *Rotylenchulus reniformis* has been reported causing great damage. In the Philippines, *R. reniformis* attacked *C. arabica*, *C. canephora* cv. Robusta and *C. liberica* var. *dewevrei* with equal severity. In India, it is an important parasite of *C. arabica*. In Brazil, it is also reported from a commercial seedling nursery and in plantations. It is also recorded on *Coffea* spp. in the Pacific island countries of New Guinea, Fiji, Tonga and Western Samoa and in Côte d'Ivoire (Campos and Villain, 2005). In Vietnam, *R. reniformis* is frequent across all coffee-growing areas. In the province of Daklak and Gia Lai, high soil densities were sometimes associated with symptoms of yellow leaf disease on both Robusta and Arabica coffee (Trinh *et al.*, 2009a; Le *et al.*, 2013; Vu



Fig. 15.16. *Coffea arabica* trees infected with *Radopholus arabocoffeae* in Vietnam; (a) seriously defoliated plant; (b) extremely deteriorated roots with important necrotic areas on the taproot. (Photograph courtesy of P.Q. Trinh.)

et al., 2014). *R. reniformis* was recently associated with, together with *Pratylenchus* sp., causing serious damage on coffee in Brazil (Salgado, personal observation). D'Souza and Screenivasan (1965) observed that in fields infested with *R. reniformis* at densities greater than 10 nematodes/50 cm³ soil, coffee did not grow well. Screening genotypes for resistance against *R. reniformis* has been conducted. Macedo (1974) found resistance in *C. canephora* cv. Guraini, whereas on *C. arabica* cvs. Mundo Novo and Catuaí, few mature females deposited eggs. No further information on the importance of this nematode and control measures is available.

Vovlas (1987) reported *Trophotylenchulus obscurus* as a pest of coffee and its widespread occurrence in São Tomé, West Africa. When feeding, *T. obscurus* introduces its anterior body portion into the peripheral layers of the cortex, and the nematode feeds from a single nurse cell, which undergoes senescence and, as a consequence, causes considerable damage to the cortical cells. Dark brown capsules containing eggs, juveniles and males can be observed on the root surface.

Many other parasitic nematode species belonging to the genera *Gracilacus*, *Caloosia*, *Criconemoides*, *Discocriconemella*, *Helicotylenchus*, *Hemicriconemoides*, *Hoplolaimus*, *Longidorus*, *Ogma*, *Paratrichodorus*, *Pratylenchus*, *Aorolaimus* (= *Peltami-gratus*), *Rotylenchus*, *Scutellonema*, *Trichodorus*, *Tylenchorhynchus*, *Paratylenchus* and *Xiphinema* have been found associated with coffee (Campos and Villain, 2005), but information on their pathogenicity, damage, yield loss and possible control measures is lacking.

Recently, two species of a new genus *Apratylenchus*, *Apratylenchus vietnamensis* and *Apratylenchus binhi*, were described from coffee in Vietnam as sedentary Pratylenchidae, with the proposal of Apratylenchinae subfamily (Trinh *et al.*, 2009b). These two species have so far been recorded only from coffee, in the Western Highlands and the centre of Vietnam, while their pathogenicity remains unknown (Le *et al.*, 2013; Vu *et al.*, 2014).

Conclusions and Future Prospects

Coffee is affected by a wide diversity of plant parasitizing nematode species that may occur simultaneously in the same region, country or plantation, creating difficulties for the implementation

of efficient control strategies. Except for some measures of nursery inspections, official measures remain too few to avoid the dissemination of infected coffee seedlings. Indeed, we observe in many countries the increasing distribution ranges of the most pathogenic nematodes, such as RKNs and RLNs. Although reliable tools (molecular and biochemical) are increasingly available for accurate species-specific diagnosis, there remains too little information on the diversity of plant nematodes parasitizing coffee in each country and/or area. Agriculture departments and/or coffee institutions need to promote monitoring studies to create the basic knowledge necessary to implement appropriate strategies for plant nematode management before and after planting. It is especially important to have accurate information on plant nematode diversity for implementing the adequate use of resistance, biological control agents and/or crop rotation. Much information on the pathogenicity of many *Meloidogyne* and *Pratylenchus* species or other species found on coffee on the various coffee cultivar germplasm (*C. arabica* and *C. canephora*) also remains lacking. This information is important for assessing or predicting the risk levels of all present nematodes.

The use of germplasm resistant to nematodes is a key pillar for integrated nematode management in coffee, whether using directly planted resistant cultivars or susceptible cultivars grafted on to resistant rootstocks. The choice of resistant germplasm may depend mostly on climatic conditions and/or targeted organoleptic quality. Coffee breeding programmes must take into account the complexity of nematode communities present in coffee fields. The use of coffee germplasm that does not offer resistance to all the damaging nematodes could be counter-productive. For example, in Costa Rica, planting cv. Sarchimor resistant to *M. exigua* but not to *Pratylenchus* resulted in substantially decreased densities of *M. exigua* but significantly higher RLN populations, which could be more damaging than *M. exigua* (Bertrand *et al.*, 1998). The *C. canephora* genome has been sequenced (Denoeud *et al.*, 2014) and *C. arabica* genome sequencing has been announced by the University of California, Davis, (2017, unpublished). Genomes of some of the major plant nematodes parasitizing coffee have also been sequenced, such as *M. incognita* (Abad *et al.*, 2008) and *P. coffeae* (Burke *et al.*, 2015).

This genomic knowledge will enable a better understanding of the interactions between nematodes and their hosts, enabling the development of new fields of durable management of plant pathogenic nematodes. Furthermore, the number of nematode sequenced genomes will likely increase during the coming years due to rapid progress in high-throughput sequencing techniques and reducing costs. In this new 'omics era', the identification of genetic or biochemical markers will facilitate resistance against nematodes. This will permit more exhaustive explorations of the wide genetic pool of wild *Coffea* spp. accessions for detecting resistance-to-nematode genes, together with other useful agronomic characters for both coffee-producing cultivars and rootstocks.

However, to ensure a durable management of selected resistances, particularly in perennial crops, complementary non-chemical control measures must be developed to reduce field inoculum pressures. Biological control is rapidly becoming an increasingly promising strategy against coffee parasitic nematodes. Moreover, thanks to metagenomic studies, much valuable information has become available during the past decade on both endophyte and rhizospheric microbiota and their essential role on key plant functions, including pest and disease defence mechanisms (Newton *et al.*, 2010; Knief, 2014; Tian *et al.*, 2015). Another important aspect to consider is that resistances to nematodes in coffee, such as against *Pratylenchus*, are quantitative or incomplete. These resistances, with a likely oligogenic or polygenic genetic determinism, are of great interest, because they may be broken with more difficulty by the pathogen. However, the expression of this kind of resistance is quite likely dependent on environmental factors (Rapilly, 1991). Durable use of these types of resistance must then be planned with appropriate coffee farming practices, such as appropriate fertilizer and soil management practices, shade in some geographical regions and, more globally, all practices that contribute to conferring an optimum physiological status on the plant, especially mitigating abiotic stress effects generated by climate change. For all these reasons, cropping under agroforestry systems seems to be the most sustainable coffee cropping option for most coffee-growing regions, and likely the most resilient to mitigate climate change.

Cocoa

Cocoa is derived from the seeds of *Theobroma cacao*, a small tree indigenous to the forests of Central and South America, belonging to the Malvaceae family, with its centre of origin in the upper Amazon Basin. In its natural habitat, cocoa grows in the understorey of evergreen tropical rainforest, often in clumps along riverbanks, where the roots may be flooded for long periods of the year. Cocoa grows at low elevations, usually below 300 m above sea level, in areas with 1000–3000 mm rainfall/year. The fruit, which botanically is a berry, usually contains 20–40 seeds, each surrounded by a pulp that is developed from the outer integument of the ovule. The action of yeasts removes the mucilage around the seeds, which facilitates subsequent handling and drying of the beans (Urquhart, 1955).

Within *T. cacao*, genetic diversity traditionally has been divided into two main groups, and a third originating from the first two (Anon., 2002). (i) The Criollo give warty, elongated pods, green or red coloured before ripeness; the white seeds give a fine and aromatic cocoa, but Criollo seeds represent just 1% of global production. (ii) The Amazonian Forastero comprise the upper and lower Amazon cultivars, the most famous of which is the Amelonado. The Forastero are widespread in Brazil, western Africa and Asia. The thick husk pods are oval, smooth and green coloured, turning yellow on ripeness. They have dark purple-coloured flat seeds and constitute most of the common cocoa and about 80% of world production. (iii) The Trinitario are hybrids originating from the first two groups and are cultivated in all cocoa-producing countries, contributing about 20% of world production. However, molecular analysis of *T. cacao* indicates that an alternative classification should be used that reflects more accurately the genetic diversity of cocoa germplasm than the traditional classification of Criollo, Forastero or Trinitario, with ten major clusters: Marañón, Curaray, Criollo, Iquitos, Nanay, Contamana, Amelonado, Purus, Nacional and Guiana (Motamayor *et al.*, 2008). This new classification is important for identifying sources of resistance and to plan heterotic combinations. Commercial cocoa cultivars continue to have a narrow genetic base, since most are derived from the traditional cultivars Criollo,

Amelonado, Nacional and, especially, Trinitario (Schnell *et al.*, 2007; Motamayor *et al.*, 2008). To ensure cocoa germplasm conservation and utilization, an important world project was initiated in 1998 after acceptance by the Common Fund for Commodities (Eskes *et al.*, 1998).

Cocoa is grown in many countries, predominantly by smallholder farmers, through latitudes between 10° north and south of the equator. The major world cocoa producers between 2010 and 2014 include Côte d'Ivoire (26% of world production), Ghana (14%), Indonesia (13%), Nigeria (6%), Cameroon (5%), Brazil (5%) and Ecuador (3%); Togo, Dominican Republic and Peru each produce 1%. Between them, these ten countries produce about 75% of the world total, around 4.2 Mt (FAO, 2017). Cocoa is a lowland crop that grows best from sea level up to altitudes of 1400 m on the equator, with temperatures of 16–34°C and rainfall of 1500–2500 mm. It reacts unfavourably to sudden changes of temperatures or humidity. The main factor limiting the growth of cocoa at higher altitudes is temperature. The daily variation of temperature should not exceed 9°C (Urquhart, 1955; Braudeau, 1970).

Cultivation techniques

Seed propagation is the most cost-efficient. Seed can be planted directly into soil (West Africa), in nursery seedbeds, in baskets or in plastic bags. Germination takes 1 or 2 weeks and seedlings are transplanted to the field at 2–6 months old. Propagation is also possible by cuttings, budgings, grafts and marcots. Spacing varies between areas; being closer in Africa and predominantly greater in the Americas and Asia at 4 m × 4 m, 3.6 m × 3.6 m and 3 m × 3 m. Shading is commonly used. Thinned natural forest for shading predominates mostly in Africa, while in America, Asia and Oceania the shade trees planted are mostly *Erythrina* spp., *Gliricidia* spp., *Albizia* spp., *Pithecolobium* spp. and *Leucaena* spp. Managing the shade conditions during the development of the crop is undertaken in some producing countries. Pruning is conducted to shape the young tree, to maintain the subsequent shape or form and to renovate or rehabilitate the tree.

Nematodes of Cocoa

Due to the susceptibility of germplasm, world cocoa production losses caused by diseases and pests are estimated at 50% (Anon., 2002). Globally, the most important plant health threats are three fungal diseases: black pod disease (*Phytophthora* spp.), responsible for 30% of global production losses, with the most virulent species, *Phytophthora megakaria*, present in western Africa (Cilas *et al.*, 1998); witches' broom (*Crinipellis pernicioso*), which occurs mainly in South America, where it has ruined the cocoa crop in many countries (Perreira, 1998); and the frosty pod disease, or monilia pod rot (*Moniliophthora roreri*), another serious disease in the Americas.

In addition, nematodes, such as *Dolichodorus* and *Meloidogyne* species, especially *M. incognita* and, to a lesser extent, *M. javanica*, are responsible for cocoa losses across the world, including sudden death of trees and growth retardation of seedlings in nurseries. Many other genera and species of root-feeding nematodes are associated with cocoa (see Campos and Villain, 2005), although the pathogenic effect for most of them has yet to be fully determined.

Meloidogyne

Meloidogyne spp. are the most important nematodes of cocoa due to their pathogenicity and wide distribution in cocoa-producing regions.

Distribution

Root knot nematodes have been recorded from cocoa since 1900 (Sosamma *et al.*, 1980), from Zaire, São Tomé, Java (Ghesquière, 1921; Cotterel, 1930; Fluitter and Mulholland, 1941), Ghana, Malawi, Côte d'Ivoire (Edwards, 1955; Luc and de Guiran, 1960; Martin, 1961), Nigeria (Caviness, 1967), Venezuela (Torrealba, 1969), Brazil (Lordello, 1968) and India (Sosamma *et al.*, 1980). *M. incognita* seems to be the most frequently recorded species (Luc and de Guiran, 1960; Sharma and Sher, 1974a). It is a common pest in West Africa (Whitehead, 1969a), including Nigeria, where this species appears as the most economically important nematode on cocoa (Badaru *et al.*, 1999). *M. incognita* is also

common in India (Sosamma *et al.*, 1980; Sudha and Sundararaju, 2002), Malaysia (Razak, 1981) and Venezuela (Crozzoli *et al.*, 2001). It is also widespread on cocoa in Brazil and the most frequently identified nematode on cocoa in the region of Espirito Santo State (Sharma and Sher, 1974a,b; Sharma, 1982). However, many of these *M. incognita* populations have been identified by their perineal patterns only, with the now known limitations of this diagnostic.

Other species of *Meloidogyne* have also been recorded on cocoa: *M. exigua* in Bolivia (Bridge *et al.*, 1982), *M. javanica* in Malawi (Corbett, 1961), Venezuela (Crozzoli *et al.*, 2001) and Central Africa (Martin, 1961), and *M. arenaria* and *M. thamesi* in Brazil (Sharma, 1979).

Symptoms of damage

Under controlled conditions, inoculated *M. incognita* causes seedling dieback, stunting, wilting, yellowing of leaves and small leaves. On the roots, tiny galls and females with egg masses can be observed. In Nigeria, seedlings of cv. Amelonado grown in soil inoculated with *M. incognita* showed symptoms from the 16th week, leading to wilting in the 24th week (Afolami, 1981; Afolami and Caveness, 1983; Afolami and Ojo 1985). Amazon cultivars also show decay symptoms, but only after the 24th week and without wilting. Sharma and Maia (1976) found that *M. incognita* caused small, rounded and elongated galls with conspicuous egg masses, and stunting on cv. Catongo. The leaf tips and margins first turn brown and become dried; this spreads across entire leaves, which are eventually shed. Infected plants appear unthrifty, with reduced height, shoot and root dry weights. In Brazil, inoculation of 10,000 *M. incognita* juveniles/plant caused significant reduction in cocoa plant growth within 17 weeks (Anon., 1975). Sharma and Maia (1975) found that *M. incognita* caused growth reductions, small internodes, thin stems, reduction of number and surface area of leaves and reduction of the root system, which had galls and fewer root hairs on cocoa cv. Catongo. Growth differences were evident 17 weeks after inoculation. Histological studies showed total disorganization of the stele, resulting in serious destruction of the xylem, phloem, pericycle and endodermis. Adult females were embedded in the cortex, with giant

cells around their heads and egg masses deposited on the root surface through ruptures in the cortex. In the field, *M. incognita* produces galls with exposed egg masses on roots, dieback and sudden death of infected plants. According to Sharma and Sher (1973), when dieback conditions occur, the trees die, while the roots remain alive and send up shoots during the following growing season. The syndrome of sudden death disease is permanent wilting; the green leaves suddenly turn yellow and brown, and then dry up but remain hanging. Jiménez-Saenz (1971) and Sharma and Sher (1973) associated the occurrence of sudden death with root knot nematode infection.

M. javanica also form galls on cocoa roots (Martin, 1961). In Malawi, young cocoa trees developed slowly in patches of soil heavily infested with *M. javanica* (Corbett, 1961). Damage symptoms were also observed on cocoa roots infected by *M. exigua* in Bolivia (Bridge *et al.*, 1982).

Nematodes can retard seedling growth in nurseries, or even kill them.

Races, means of dissemination, other hosts and economic importance

No attempt appears to have been made to determine the intraspecific variation of *M. incognita* or *M. arenaria* populations infecting cocoa.

M. javanica, *M. incognita* and *M. arenaria* have wide host ranges (Ponte, 1977; Nickle, 1984), and in many instances the commonly used shade plants, such as banana, may become a source of inoculum to cocoa (Sosamma *et al.*, 1980). Corbett (1961) recommended the replacement of banana as a shade crop to reduce nematode infections on cocoa in Malawi.

As for coffee or any other perennial crop, nematode-infected seedlings from nurseries remain the principal pathway of nematode dissemination to plantations. Into and between closed cocoa plantations, runoff water from infested plots may also spread nematodes.

Although there is no reliable data on cocoa yield losses caused by nematodes, evidence indicates they can be important constraints. The sudden death of cocoa plants in the field has been associated with *Meloidogyne* spp. in many cocoa production areas, where they are a likely limiting factor to productivity and have an economic impact.

Other nematode parasites of cocoa

Many other root-feeding nematodes have been identified on cocoa (see Campos and Villain, 2005), but with limited information on their importance. The lesion nematode *P. brachyurus* has been widely associated with cocoa in Bahia, Brazil (Sharma and Sher, 1973); it also occurs in western Africa (Luc and de Guiran, 1960). In Java, *P. coffeae* infects roots of cocoa (Fluitter and Mulholland, 1941). Sudha and Sundararaju (2002) found *P. coffeae* on cocoa in Kerala state, India, and Kumar *et al.* (1971) reported the multiplication of this nematode on cocoa in glasshouse experiments. *P. coffeae* is also reported on cocoa in Indonesia (Siddiqi, 1972), and *P. zaei* occurs in Venezuela (Crozzoli *et al.*, 2001).

Sharma (1971) associated dieback and death of nursery plants with the presence of *Dolichodorus* sp., since identified as *Dolichodorus minor*. The entire root system was reduced, blackened, with disintegrated cortex and bead-like gall formation. The galled portion was reddish-brown and hard. In Para State, northern Brazil, *D. minor* was one of the most common nematodes infecting cocoa; *D. minor* was also reported on cocoa in south-eastern Costa Rica (López, 1994).

Helicotylenchus spp. are widespread on cocoa in South America and Asia, and *Helicotylenchus pseudorobustus* reproduced on cocoa in Liberia (Lamberti *et al.*, 1992). *Helicotylenchus dihystra* was reported in Bahia State, Brazil, as the most widespread species on cocoa, occurring in 70% of samples (Sharma and Lordello, 1982). Luna (1976) and Campelo and Galli (1980) demonstrated the pathogenicity of this nematode on *T. cacao*. A significant reduction was observed in root dry weight and leaf number 188 days after inoculation of various levels over 20 nematodes/plant; stunting and significant decrease of root dry weight in 20-day-old inoculated seedlings was also observed.

Management

In perennial crops such as cocoa, nematodes that survive the control practices have time to recover and build up again to destructive levels. Hence, the most efficient control strategies are: (i) to produce nematode-free seedlings; and (ii) to cultivate in soils or areas where nematodes are absent. Soil to be used in the nursery can be

disinfested by steam or by solarization (see above under Coffee). Using soil collected from areas free of the most pathogenic nematodes, such as RKNs and RLNs, is a good measure for the production of nematode-free seedlings. However, some efficiency of chemical nematicides for decreasing nematodes has been demonstrated for cocoa (Campos and Villain, 2005). Efficiency of nematicides may be of limited duration, however, while the environmental impact and health hazards of using nematicides needs to be considered. The tendency is now oriented more to the use of germplasm with resistance to nematodes and other major pests and diseases, and to alternative control measures such as biological control and the use of organic amendments within an integrated management strategy (Orisajo, 2009). The most promising control measure is the use of resistant germplasm, as currently achieved against fungal diseases. Resistance to *M. incognita* has been identified in five genotypes of cocoa in Nigeria (Badaru *et al.*, 1999), with further screening of *T. cacao* germplasm for resistance to *M. incognita* undertaken in Nigeria (Afolami and Ojo, 1985). In Brazil, 12 cacao hybrids were tested for resistance to *M. incognita* and showed a reproduction factor of the nematode from 6 for the hybrid TSH565 × S1C802 to 39.6 for the hybrid SIAL70 × SIAL88. This hybrid (TSH565 × S1C802) also presented the lowest galling index (Anon., 1976). In Niger, differences in susceptibility to *M. incognita* were observed among four cultivars in glasshouse inoculation tests (Asare-Nyako and Owusu, 1981). A high degree of resistance to *M. incognita* was exhibited by three clones, LCTEEN, T12/11 and AMAZ 15-15, in field experiments in Nigeria (Okeniyi *et al.*, 2015).

Orisajo *et al.* (2012) demonstrated in nurseries and fields in Nigeria that a poultry litter soil amendment not only enhanced soil fertility but also increased microbial diversity, and also depressed plant parasitic nematode densities. Okeniyi *et al.* (2016) also found in Nigeria that the application of cocoa pod husk and/or neem leaves as an organic amendment significantly reduced the densities of a range of cocoa parasitic nematodes present in the field, especially the most frequent *Meloidogyne* spp. Care must be taken with the selection of shade plants, avoiding trees susceptible to RKNs and RLNs, such as, for example, *Leucaena leucocephala* (= *Leucaena*

glauc) and banana (Corbett, 1961; Sosamma *et al.*, 1980).

Methods of diagnosis

Root galling in most cases will be helpful to diagnose the presence of *Meloidogyne* species on cocoa plants. However, the extraction of juveniles of those nematodes from roots can help to confirm their presence and identification. Species-level identification for *Meloidogyne* should be achieved by esterase electrophoresis and by using species-specific molecular markers (see Chapter 4, this volume). For *Pratylenchus* species diagnosis, see also the section 'Nematode Parasites of Coffee' above. Morphological observation, together with morphometrics and molecular phylogenetic inference, should be used to determine *Helicotylenchus* populations accurately at species level (Subbotin *et al.*, 2015). For *D. minor*, identification is made by morphological evaluation and morphometrics.

Conclusions and Future Prospects

The sudden death syndrome of cocoa trees, particularly of seedlings linked to RKN presence, and especially to *M. incognita*, appears to be extending, and is now reported from Bahia State, Brazil, Nigeria and some other important cocoa-growing regions. Since the potential pathogenicity of some nematodes, especially *M. incognita*, can be high, cocoa growers must be aware of this threat. As for coffee, prophylactic measures should be taken in nurseries to avoid the dissemination of infected seedlings, preferably through certification schemes. The exclusion approach for preventing damage is more cost-effective in the long term, safer, and more efficient, especially for perennial crops such as cocoa or coffee.

Emphasis should, in future, be placed on more accurate monitoring at the species level and on the estimation of yield losses and distribution of the most damaging nematodes for cocoa in the different cocoa-growing regions, to obtain a better picture of their economic importance and distribution. Many countries have the potential to increase cocoa production, but a profitable crop will require good management of the various agronomic aspects, including nematode diseases. Larger markets and a greater competition on a worldwide basis require greater efficiency of production at lower prices, minimizing the costs and risks involved. At this time, cocoa growers and professionals have given more importance to the three major fungi diseases cited above, but, even though their damage may be more progressive and less conspicuous, nematodes can increase the cost of cocoa production significantly and decrease yields. To date, breeding programmes have prioritized resistance to black pod disease (*Phytophthora* spp.), witches broom (*C. pernicios*), monilia pod rot (*M. roreri*) or viral swollen shoot disease, but not resistance to nematodes (Nguyen-Ban, 1996; Eskes *et al.*, 1998; Knight, 1998; De Franqueville, 2001). The intensification of cocoa cropping and a focus on controlling the main diseases through resistant germplasm, without due vigilance to nematodes, could result in nematode issues rising to the foreground, without it being noticed, and causing economic impact. It is important, therefore, that cocoa breeders consider including resistance to nematodes in cocoa breeding programmes in regions where highly pathogenic nematodes such as *M. incognita* or *P. coffeae* are present. The genome of *T. cacao* has been sequenced (Argout *et al.*, 2011), allowing new perspectives for identifying sources of multiple resistances to plant parasitic nematodes and other pests and diseases.

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16 Nematode Parasites of Tea*

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Tea is a beverage crop with two extreme varieties including the small-leaved China type and the large-leaved Indian or Assam type, both of which belong to the same species, *Camellia sinensis*. Commercial tea populations are polymorphic in origin, derived from *C. sinensis* *Camellia assamica* var. *assamica* and *C. assamica* var. *lasiocalyx*, or the hybrids of these different varieties.

Tea is grown presently at latitudes from 27°S (Corrientes, Argentina) to 43°N (Georgia, former USSR), as well as from mean sea level up to an altitude of 2300 m. The tea crop requires well-drained acid soils with a pH range of 4.5–5.5 and reasonably well distributed rainfall, totalling not less than 1000 mm/year.

Cultivation techniques

The population of tea bushes in old tea fields is about 7000/ha, and in many fields the plant population is far below this number, due to extensive casualties. The plant population density in the newly planted areas is around 13,000, usually planted along the contour.

When allowed to grow freely, the tea plant could grow to a large tree, attaining a height of around 12 m or more. For purposes of commercial exploitation, the plant is pruned regularly, to be

maintained in the form of a bush at a height of around 90 cm.

The unit that is harvested is the tender flush, usually comprising two or three leaves and a bud, and these units are generally harvested at weekly intervals, depending on growth rates.

The average yield of tea could range from as low as 500 kg/ha to as high as 6000–7000 kg/ha of made tea/year (which corresponds to ~1600–32,500 kg of green leaf/ha/year), depending on the climate, the specific cultivar planted, the presence of various pests and diseases (which is more severe in the Asian countries, compared to the African countries), and other socio-economic factors. Though broadly similar, the agricultural and manufacturing practices could vary in the different tea-growing areas of the world.

Nematode Species Encountered in Tea

The factors generally limiting nematode reproduction and survival and establishment in a given specific location are known to be very much dependent on the soil environment (Gnanapragasam, 1988, 1994a). Due to the wide variability in soil types and climatic conditions under

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which tea is cultivated on a commercial scale, the complex of nematode populations that attack the tea plant varies very widely, and the intensity of attack of the respective species and the degree of the induced pathogenicity could also vary correspondingly. Furthermore, investigations with respect to damage caused by pathogenic nematodes to the tea crop is as yet limited to a few countries only.

Several species of plant parasitic nematodes have been recovered from the roots of tea and/or from the rhizosphere of tea plants in the different tea-growing areas of the world. These include: *Meloidogyne arenaria*, *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne hapla*, *Meloidogyne thamesi* and *Meloidogyne brevicauda*, *Pratylenchus loosi*, *Pratylenchus brachyurus*, *Pratylenchus penetrans*, *Radopholus similis*, *Hemicriconemoides kanayaensis*, *Hemicriconemoides californianus*, *Hemicriconemoides mangiferae*, *Helicotylenchus dihystera*, *Helicotylenchus erythrinae*, *Hoplolaimus* sp., *Rotylenchus* sp., *Rotylenchulus reniformis*, *Tylenchus* sp., *Hemicycliophora longicaudata*, *Hemicycliophora typica*, *Xiphinema americanum*, *Xiphinema radicicola*, *Hoplolaimus* spp., *Scutellonema* sp., *Aphelenchoides* sp., *Paratylenchus curvatus* and *Paratylenchus lepidus* (Hutchinson and Vythilingam, 1963b; Sivapalan, 1972; Chandel, 1995; Chen *et al.*, 2007; Bhattacharya *et al.*, 2012a, 2013; Orisajo, 2012; Kibet *et al.*, 2013).

However, no positive evidence of pathogenicity has been established with respect to the majority of these nematodes. The species that cause economic damage to tea include: *Pratylenchus* spp., *R. similis*, *M. arenaria*, *M. incognita*, *M. javanica*, *M. hapla*, *M. thamesi*, *M. brevicauda* and *Hemicriconemoides kanayaensis* (Gnanapragasam, 2014) (Table 16.1).

Pratylenchus loosi

Species of *Pratylenchus* are known to attack tea growing in almost all parts of the world. Among these, *P. loosi* is the most serious pest in Sri Lanka (Gadd, 1939; Gadd and Loos, 1946; Loos, 1953a; Sivapalan, 1972). This species of nematode is also recorded as a serious pest of tea in Japan (Kaneko and Ichinohe, 1963; Takagi, 1967, 1969, Ohta *et al.*, 2014) and Iran (Maafi, 1992). It is also reported from West Bengal, India (Mukherjee and Dasgupta, 1982), Bangladesh

(Khan *et al.*, 2003; Mamun *et al.*, 2011), Kenya (Orisajo, 2012), Taiwan (Wu *et al.*, 2002, Chen *et al.*, 2009) and Korea (Park *et al.*, 2002).

In Sri Lanka, *P. loosi* is widely distributed among tea fields at all altitudes. However, damage to tea is mostly serious at elevations of 900–1800 m, where severe damage and crop loss occur in mature tea and newly planted young fields, as well as in nurseries (Hutchinson and Vythilingam, 1963a; Sivapalan, 1972; Gnanapragasam, 1986a). As a consequence of its distribution and pathogenicity to high elevation tea areas, it is commonly referred to as the ‘up-country species of nematode’. With the current changes in weather pattern, serious outbreaks are occurring at some locations at lower elevations as well.

In contrast, in Japan, where tea is cultivated at altitudes of 0–300 m, damage to tea by this species occurs at all locations in view of the fact that this country is located in the cooler temperate zone (Takagi, 1969; Gotoh, 1976). During the past few decades, the intensity of investigations on the nematode problem in tea has dwindled in Japan.

P. loosi is also known to cause damage to tea in China (Chen and Chen, 1982; Li, 1985) and Taiwan (Wu *et al.*, 2002; Chen *et al.*, 2009), but to date, a proper survey has not been carried out and, as such, the distribution and extent of damage are not well known.

In Darjeeling, India, *P. loosi* was reported in 1982, but no pathogenicity trials have been carried out (Mukherjee and Dasgupta, 1982).

In Bangladesh, this nematode has been observed to cause symptoms of damage to tea in nurseries only. Nursery soils are, therefore, regularly checked for this species and treated (Khan *et al.*, 2003). Despite such observations, no further attempt has been made to assess the distribution and possible damage in mature tea.

P. loosi was recorded in Korea in 2000, when it was isolated from the roots and rhizosphere of tea in the districts of Yeongam-gun, Jeollanam-do and Namjeju-gun and Jeju-do. No pathogenicity trials have yet been reported (Park *et al.*, 2002).

In Iran, *P. loosi* was reported in 1992 in the tea gardens in Amlash, Guilan Province, in the north of Iran near the Caspian Sea, which has a subtropical climate (Maafi, 1992). Studies carried out later revealed the presence of this nematode in the entire region of Guilan province,

Table 16.1. Distribution of nematodes causing economic damage to tea in tea-growing regions.

Nematode sp.	Argentina	Australia	Bangladesh	China	India N	India S	Indonesia	Iran	Japan	Kenya	Korea	Malawi	Nigeria	Sri Lanka	Taiwan	Zimbabwe
<i>Pratylenchus loosi</i>			+		+			+	+	+	+			+	+	
<i>Pratylenchus brachyurus</i>		+			+							+				
<i>Pratylenchus penetrans</i>					+											
<i>Radopholus similis</i>				+			+							+		+
<i>Meloidogyne brevicauda</i>					+	+								+		
<i>Meloidogyne incognita</i>	+		+	+	+		+	+	+	+		+	+	+	+	+
<i>Meloidogyne arenaria</i>				+	+			+				+		+		+
<i>Meloidogyne javanica</i>				+	+	+	+	+				+		+		
<i>Meloidogyne hapla</i>				+	+			+								+
<i>Meloidogyne thamesi</i>				+												
<i>Meloidogyne acrita</i>				+												
<i>Hemicriconemoides kanayaensis</i>				+					+						+	

ranging from the lower to the higher elevations (Hajieghrari *et al.*, 2005). Since then, intense studies have been carried out in this country to control and arrest the spread.

Symptoms of damage

Typical symptoms of injury caused by *P. loosi* in both young and mature tea in the field include patches of unthrifty tea (Fig. 16.1), with the affected plants showing spindly growth with sparse foliage. The leaves are dull, brittle and pale. These symptoms are brought about by an altered rate of uptake of essential nutrients by the damaged root system (Fig. 16.2). The heavily infested plants also have a tendency to start the reproductive phase by flowering and setting fruit prematurely. Examination of the roots of such infested plants shows a marked reduction in the growth of feeder roots. The remaining roots appear brown and dried up when compared with normal healthy roots that are succulent and whitish in colour. Dark brown necrotic patches

or lesions of varying size are displayed on the peeling bark of the larger storage roots (Fig. 16.3). The heavily infested plants either recover very poorly from pruning, remain as unthrifty 'passengers', or fail to recover at all and die (Gadd, 1939; Visser, 1959; Sivapalan, 1967a, 1972).

Biology and life cycle

Like other *Pratylenchus* species, *P. loosi* is a migratory endoparasite invading the epidermis and cortex of the host plant, causing necrosis and cavities. Although the cell walls were thickened, there was no evidence of damage in the vascular cylinder (Inserra *et al.*, 1980). They move into the soil in search of fresh feeder roots when the parasitized roots are severely damaged or become over-parasitized. Thus, it is very common to encounter large populations in the soil or in the rhizosphere of infested bushes at a depth of 15–25 cm. The population density, however, is usually lower near a dying or heavily damaged plant in the field, as most of the roots would have



Fig 16.1. A tea field heavily infested with *Pratylenchus loosi*, showing unthrifty tea bushes and casualties. (Photograph courtesy of N.C. Gnanapragasam.)



Fig. 16.2. Stunted tea plant with feeder roots damaged by *Pratylenchus loosi* (right) and uninfested healthy plant (left). (Photograph courtesy of N.C. Gnanapragasam.)



Fig 16.3. Storage root of tea with lesions formed by *Pratylenchus loosi*. (Photograph courtesy of N.C. Gnanapragasam.)

decayed. The nematodes are mostly attracted to the growing parts of the roots, where they penetrate and enter near the root tips. According to Seinhorst (1977), it takes the nematode 45–48 days to complete its life cycle, comprising 15–17 days for the eggs to hatch, 15–16 days as juveniles and 15 days as adults before egg laying. Takagi (1969) and Seraji *et al.* (2007) also agreed broadly with the above observations. Egg laying was found to be delayed in the absence of males (Gadd and Loos, 1941). The optimum temperature range for the highest population build-up and visual symptoms was found to be at soil temperatures of 18–24°C (Sivapalan and Gnanapragasam, 1975), while according to Seraji *et al.* (2007), the optimum temperature for reproduction *in vitro* on carrot disc cultures was 20–21°C.

Pathotypes (biological races)

Morphological and morphometric studies carried out in Sri Lanka and Iran on the males and females of *P. loosi* have revealed the possible existence of different pathotypes/strains (Pourjam

et al., 1997, 1999; Mohotti, 1998; Mohotti *et al.*, 1998, 2002). Although five populations of this species collected from tea soils from geographically different areas, including Iran, Japan (Kagoshima and Shizuoka prefecture) and Sri Lanka (Passara, north-east monsoonal zone, and Talawakelle, south-west monsoonal zone), respectively, showed morphometrical similarities, observations made under the electron microscope showed distinct variation in the head and tail regions. The intraspecific variability thus observed in the *P. loosi* populations may be attributed to variation in the geographical area, host nutrition and the origin of the nematodes. The *P. loosi* populations were found to be conspecific with each other and demonstrated a *P. loosi* species complex (Mizukubo, 1998; Mohotti *et al.*, 2002).

Comparative d2/d3 LSU-rDNA sequence studies have revealed a closer relationship between the Iranian and Sri Lankan isolates of *P. loosi* and distinct differences from isolates from native plants from Florida (Hajieghrari *et al.*, 2007). Detailed morphological and morphometric studies carried out by Mohotti (1998) raised doubts regarding the identity of *P. loosi* recovered from non-tea hosts in Florida.

Survival and means of dissemination

P. loosi is known to survive in host-free soils in the lesions of the larger, old storage roots of tea that are left uncleared, following the uprooting of old tea fields, for as long as 3 years.

One of the most important means of spread of *P. loosi* among tea areas is by the dissemination of infested plants to fields from contaminated

nurseries. The spread of nematodes could also occur through: (i) the movement of infested soil and water – poor soil conservation measures adopted in infested areas, including the use of weeding implements that tend to loosen the soil, and inducing erosion and washing down of contaminated soil into areas hitherto uninfested; (ii) uprooting of old tea fields sometimes carried out from the bottom of the slope upwards, thus exposing the newly planted young tea at the bottom to reinfestation from infested old tea still remaining above; and (iii) the use of contaminated irrigation water in nurseries (Gnanapragasam, 1985a, 1989).

Environmental factors affecting pathogenicity

The severity of damage to tea is dependent on the interaction of various factors such as: (i) the prevailing climatic conditions; (ii) the type of soil in which the tea is growing; (iii) cultural practices; and (iv) the age and vigour of the plant (Gnanapragasam, 1988).

CLIMATIC FACTORS: the distribution of *P. loosi* is determined mainly by soil temperature and soil moisture. The highest population is encountered at altitudes with soil temperatures of 18–24°C. Obvious pathogenicity symptoms are also observed in this temperature regime (Sivapalan and Gnanapragasam, 1975; Gnanapragasam and Manuepillai, 1984). At temperatures above and below this range, the rate of population build-up is less, and consequently, damage to tea is also reduced (Sivapalan, 1972).

With recent changes in climate, including increased rainfall and decreased sunshine in lower elevations of Sri Lanka, populations of this species are reported to be spreading to hitherto uninfested areas at lower elevations (Mohotti, 2009).

The results of earlier detailed surveys have revealed that the largest population of this species of nematode is encountered in areas with high and well distributed rainfall, and this determines the severity of damage within the same altitude (Hutchinson and Vythilingam, 1963a), a trend that holds true now as well in the ideal soil temperature zone (Gnanapragasam, personal observation). Seraji *et al.* (2007), however, found that soil temperature played a more important role than rainfall in population build-up

of this nematode in Iran. A marked periodic fluctuation in population levels is also observed during the year, and this variation is correlated to the rainfall pattern, as well as to soil temperature (Sivapalan, 1972) (Fig. 16.4).

TYPE OF SOIL: nematode damage is known to vary with the type of soil (soil texture) as well as the physical condition of the soil. Damage caused by *P. loosi* was observed to be most severe in clayey, poorly-drained soils (Sivapalan, 1971).

Under poor soil conditions, the rate of replenishment of roots damaged by nematodes is very much curtailed, resulting in the rapid deterioration of the root system, with the consequent restricted uptake of nutrients, and the plants soon turn out to be mere 'passengers'. Choshali *et al.* (2013) reported a direct correlation of soil pH to mean population density of nematodes per gram of root, with the highest population level reported to be at a pH of 3.5–4.5. However, according to Gnanapragasam (1987a), experiments carried out on 600 tea field soil samples indicated no direct correlation between soil pH and the numbers of *P. loosi*. However, pH had only an indirect effect wherein, with high acidity, tea plants were subject to severe damage from an even lower population of nematodes than those in soils with an optimum pH.

INFLUENCE OF CULTURAL PRACTICES: due to the large genetic variability in seedling tea fields, the pattern of distribution of nematode infestation in such fields is highly clustered. When such old fields are replanted to the genetically uniform, high-yielding, vegetatively propagated varieties, the spread of infestation could become more uniform, depending on the susceptibility ratings of specific cultivars.

The presence of shade trees and green manure crops among tea fields, which form part of the normal cropping pattern, also influences the distribution pattern and the intensity of build-up of this species of nematode, provided they too happen to be good hosts to this species of nematode (Sivapalan, 1972; Gnanapragasam, 1987b).

Alternative hosts

The presence of other hosts in the vicinity of tea fields also regulates the population levels of *P. loosi*. The presence of crops such as *Tephrosia*

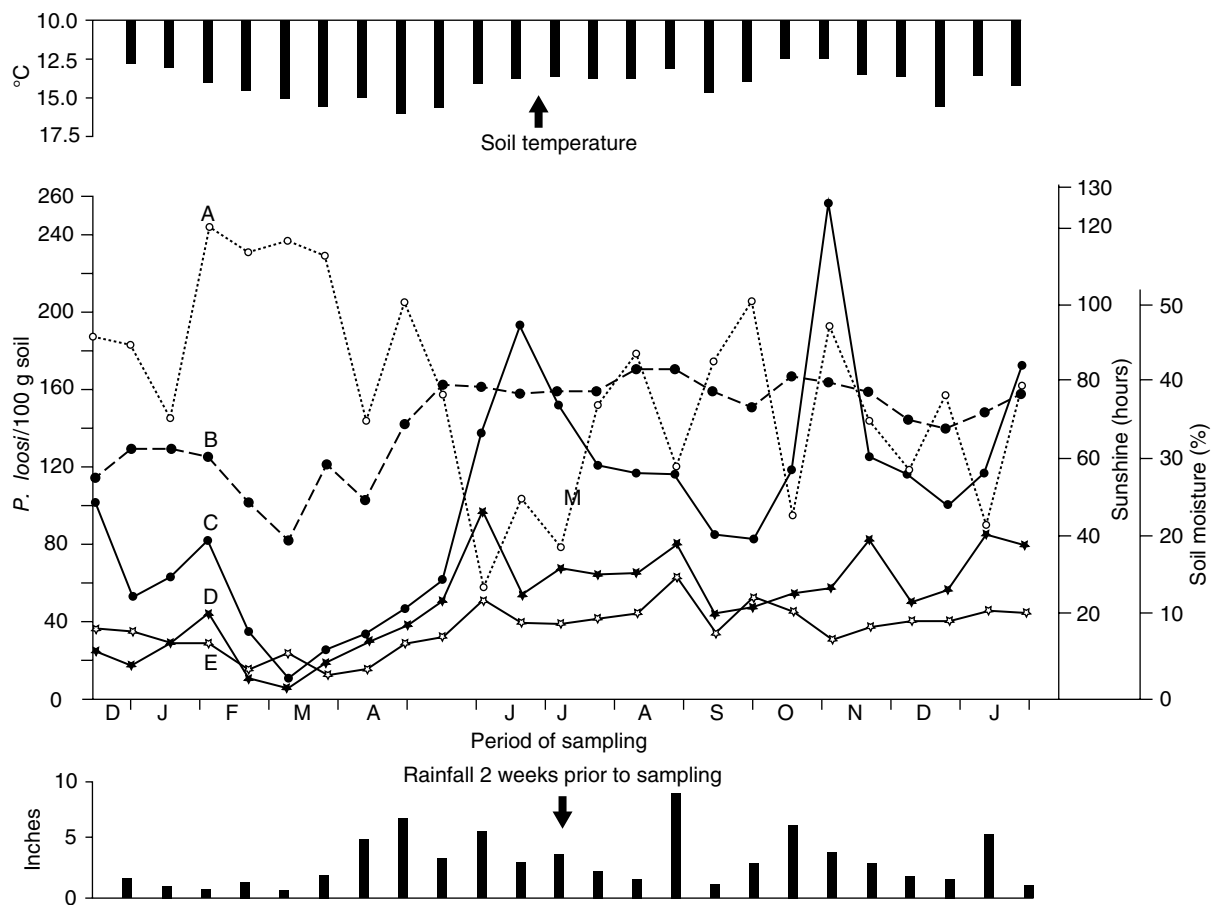


Fig. 16.4. Soil population fluctuation of *Pratylenchus loosi* at varying depths (C = 15 cm, D = 30 cm, E = 45 cm) during different times of the year, as determined by soil temperature, rainfall pattern and sunshine (A) and soil moisture (B). (From N.C. Gnanapragasam.)

vogelii, *Sesbania cinerascens*, *Cassia elata* and *Acacia* spp., as well as certain weeds, increases the incidence of this nematode species in tea fields (Visser, 1959; Sivapalan, 1972; Gnanapragasam, 1987b; Gnanapragasam *et al.*, 1989). Grasses such as Guatemala (*Tripsacum laxum*) and Mana (*Cymbopogon confertiflorus*) are non-hosts and thus do not help in the build-up of this species of nematode. On the other hand, grasses such as *Eragrostis curvula*, as well as specific plants such as *Tagetes* spp. (marigold), *Arachis pintoi*, *Tithonia diversifolia* (wild sunflower), *Wedeliya trilobata*, *Vetiveria zizanioides* (vetiver), *Adhatoda vasica*, *Ricinis communis*, *Azadirachta indica*, *Madhuca indica*, *Sambucus javanica*, *Plectranthus zeylanicus*, *Indigofera teysmannii*, *Eupatorium inuliformes*, *Calliandra calothyrsus* and *Crotalaria anagyroides* help to reduce the population (Visser and Vythilingam, 1959; Hutchinson, 1962; Kerr, 1963a; Sivapalan, 1972; Gnanapragasam, 1981, 1995, 1997).

In Iran, tea has so far been found to be the main host of this nematode. However, among several tested weeds, *P. loosi* was recovered from the roots of basket grass (*Oplismenus compositus*), which is one of the most important weeds in tea areas of Iran (Mirghasemi and Seraji, 2010).

Although *P. loosi* has been reported in coffee (*Coffea arabica* cv. Catuai) in Quetzaltenco, Guatemala (Anzueto and Sarah, 1992), in Sri Lanka, in areas where coffee is intercropped with tea, no build-up of populations has been encountered so far. It is possible that the species found in Guatemala is a different pathotype to that encountered in Sri Lanka.

P. loosi has also been reported in several other hosts, including the roots of *Sorghum vulgare* (Baujard, 1986), groundnut, millet, cowpea in Senegal, in the Saheline Province of West Africa (Baujard *et al.*, 1990), citrus in Iran (Divsalar *et al.*, 2012) and citrus, *Hibiscus sinensis* and okra in New Delhi (Sethi and Swarup, 1971; Nath *et al.*, 1975), banana (M.R. Siddiqi, St Albans, UK, 1995, personal communication), cotton, pasture grasses such as bahia grass (*Paspalum notatum*) and maiden cane (*Panicum hemitomon*) (Inserra *et al.*, 1996), apple (*Malus domestica*) and grape (*Vitis vinifera*) in New South Wales, Australia (McLeod *et al.*, 1994), citrus and pear in Japan (Gotoh, 1974) and mango (*Mangifera indica*). However, detailed studies carried out on some of the populations collected

from the non-tea hosts has revealed extensive morphological and morphometric variations raising doubts as to their actual identity (Mohotti, 1998). Doubt has also been raised as to the identity of *P. loosi* collected from non-tea hosts (M.R. Siddiqi, St Albans, UK, 1998, personal communication). Even if some of these species collected from non-tea hosts take on tea, they would not cause any threat to the infestation and spread of nematodes in tea fields as these plants are not normally grown in the vicinity of tea areas.

Disease complexes

Very limited work has been carried out on disease complexes involving nematodes parasitizing tea. The occurrence of a soft root rot disease on mature tea roots, leading to death of the affected plants during dry weather, is a disease complex formed by *P. loosi* and a group of three fungi (*Purpureocillium lilacinum*, *Paecilomyces* sp. and *Absidia corymbifera*) (Arulpragasam, 1981). This condition is reported to be brought about by many factors, the primary cause being the predisposition to infestation with *P. loosi* (Arulpragasam and Addaickan, 1983). Enhancement of pathogenicity due to the interaction of soil-borne fungi and *P. loosi* has also been reported in Iran, brought about by a complex of *P. loosi* and *Rhizoctonia solani*, *Fusarium proliferatum*, *Fusarium pallidorozeum* or *Sclerotium rolfsii* (Hoseini *et al.*, 2010).

A disease complex involving nematode–insect interaction causing yield decline has been observed recently in some of the mid-elevation tea areas of Sri Lanka (200–1000 m). The most serious pests of tea in this region include the insect pest, shot-hole borer (*Euwallacea fornicatus*), and the plant parasitic nematode *R. similis* and, at the upper limit of this elevation range, *P. loosi* as well. Both the above individual nematode species and the insect pest could by themselves cause severe damage to tea when the population level increases beyond their respective damage threshold level. However, when either of the nematode pests and the insect pest attack the tea plant simultaneously, serious economic damage is brought about at levels well below the respective damage threshold level, and the symptoms of damage also become accentuated (Gnanapragasam, 2002a).

Slow decline of a nematode-tolerant tea cultivar

There have been several instances of slow decline of tea, ultimately leading to death of the affected tea bushes, especially following pruning in the high-elevation tea areas and in a few of the mid-elevation tea areas of Sri Lanka. In all of these instances, the affected tea cultivar is TRI 2025, a popular high-yielding tea cultivar in Sri Lanka, which is known to be only weakly susceptible to *P. loosi*, that has reached the age of about 20 years or more. Although the above-ground symptoms greatly resemble the symptoms brought about by 'soft root rot' disease, the typical pulpy soft appearance of the roots is not present. The typical whitish fungal mycelia in the 'soft root rot' affected bushes are also not evident among the bushes affected by the slow decline of tea. Unlike in the case of 'soft root rot' of tea, where the bushes died after 3–6 months, the death of bushes in this case occurs only after some years. Detailed investigations have revealed that the observed slow decline is brought about by a long-term protracted infestation with *P. loosi* in high-yielding tea fields in which the tea bushes have been subject to other forms of environmental stress (Gnanapragasam, 2002b).

Economic importance and population damage threshold levels

Detailed assessment on crop losses in tea caused by plant parasitic nematodes has been carried out almost entirely in Sri Lanka. Although *P. loosi* has been recovered from several locations, significant damage to tea has been observed mostly at elevations of 900–1800 m. The decline in yield in such areas, although earlier estimated to be in the order of around 225–350 kg of made tea/ha/year (Gadd, 1939; Visser, 1959), could range between 4 and 40% of loss in crop depending on the type of cultivar planted, prevailing climatic conditions, population density of nematode, age and vigour of affected tea bushes, type and condition of soil and pH of soil, etc. (Gnanapragasam, 1988). The extent of damage is, however, far greater in infested young tea clearings and nurseries, where casualties could range from 60 to 100%, especially when the required sanitary measures are not followed. Of about 55,000–60,000 ha of high-elevation tea

areas in Sri Lanka, approximately 40–60% are known to suffer obvious damage by this species of nematode. Economic losses caused could be experienced in the remaining infested tea areas as well, but such losses have not yet been ascertained, as in most of these areas the observed decline in yield is brought about by more than one factor.

It is difficult to estimate with any precision the population damage threshold of any species of nematode causing an economic loss to a given crop, as this is compounded by an interaction with other environmental factors. In general, a tea plant that is already under stress due to other causes readily succumbs to infestation by even a low population. However, in experiments carried out under controlled conditions in the greenhouse, the damage threshold of *P. loosi* was estimated to be 40 nematodes/100 g of soil, at 24°C, which is the mean temperature of areas between the elevation range of 900 and 1800 m (Gnanapragasam and Manuelpillai, 1984).

Pratylenchus brachyurus

Unlike *P. loosi*, *P. brachyurus* only causes damage to young tea (1- to 3-year-old plants). In north-east India, this species has been detected in the plains of Assam (Basu, 1968; Mukherjee and Dasgupta, 1982) and Tripura (Bhattacharya *et al.*, 2013). It has also been reported in Malawi (Corbett, 1967), Vietnam (Ryss and Fam Tkhan' Bin', 1989). In Sri Lanka, although *P. brachyurus* was detected in the rhizosphere of *Albizia moluccana* trees in the mid-altitude tea areas, the neighbouring tea plants were not infested (Gnanapragasam, 1991). In Queensland, Australia, where tea was planted relatively recently, this species has been found to attack tea seedlings up to the age of 12 months. Thereafter, there is no evidence of pathogenicity (P.C. O'Brien, Australia, 1988, personal communication).

A similar observation has also been made in Malawi (Corbett, 1967). The damaged plants are stunted and unthrifty and show characteristic nutrient-deficiency symptoms. This nematode attacks mainly the feeder roots, and occasionally the taproot as well. During its feeding activity, it moves deep into the root tissue, causing the formation of dark red lesions on the epidermal layer. This species is reported to survive long

periods of drought, during which time they remain quiescent (Basu, 1968).

Pratylenchus penetrans

This is a migratory endoparasite found mostly in temperate and subtropical regions. This species has been reported in tea from Uttar Pradesh, India, only (Jauhari and Madan, 2001). Further investigation is necessary to confirm the importance of this finding. Laboratory experiments carried out to study the effect of different soil temperatures on population build-up in sandy-loam and sandy-clay soils have revealed maximum populations at 25°C in sandy-clay and at 30°C in sandy-loam soils, and the lowest populations at 35°C in sandy-clay and at 35°C in sandy-loam soils (Madan and Jauhari, 2007). Soil moisture was also reported to have a positive effect on population build-up (Jauhari and Madan, 2001).

***Meloidogyne* spp.**

Meloidogyne species are the most commonly encountered nematodes in tea in the different tea-growing areas of the world. Most of these species attack only young nursery plants. In Sri Lanka, mature tea plants were observed to become totally immune, with the plants developing resistance at 12–14 months of age. In some countries, infestation has been reported up to 3 years of age. The only exception is *M. brevicauda*, which is known to attack mature tea very severely.

Distribution

The first report of root knot nematode infestation in young tea was from south India, where they were found to infest large numbers of tea seedlings (Barber, 1901). In Sri Lanka in 1928, Stuart-Light ascribed large-scale failures in tea nurseries to infestations caused by root knot nematodes. Since the 1960s, tea has been propagated by vegetative means, rather than from seeds, and infestation of nursery plants by this species of nematode is seldom encountered in this country. The species that are known to infest young tea in Sri Lanka and north-east India

include *M. incognita*, *M. javanica* and *M. arenaria*. On the other hand, *M. hapla* has rarely been found to infest tea (Banerjee, 1967; Gnanapragasam, 1985b). Bhattacharya *et al.* (2013), however, reported *M. hapla* to be more prevalent in tea areas, followed by *M. incognita* in Tripura, India. Root knot damage to tea was shown to be more abundant at high altitudes in India than at lower elevations (Basu and Roy, 1976).

In Malawi in 1960, almost all the tea estate samples were infested with *M. javanica* (majority of areas), *M. incognita* and *M. arenaria*. As has been reported from other countries, such infested samples were all from tea nurseries (Martin, 1960, 1962). In Zimbabwe, species encountered include *M. incognita*, *M. arenaria* and *M. hapla* (Keetch and Buckley, 1984). Severe nematode attack on tea by *M. incognita* in Kenya was reported in 2002 (Otieno *et al.*, 2002). In Nigeria, *M. incognita* was observed to cause decline symptoms to all tested tea clones and the worst affected ones were the china clones (Orisajo, 2012). In China, the incidence of root knot nematode damage was found to be about 90% in tea seedlings, and the death rate was estimated at 40% in the seriously affected nurseries. About seven to eight population peaks were reported for the juveniles within tea root (Rong *et al.*, 1984; Yao and Yu, 1993). In Yunnan Province, *M. incognita*, *M. javanica* and *M. arenaria* have been reported (Yu and Xia, 1987). In Zhejiang Province, *M. thamesi* has also been found in addition to the other three species (Huan, 1984; Rong *et al.*, 1984). In both these provinces, *M. incognita* was found to be more abundant than the other species (Yu and Xia, 1987). In addition to the above species, in China *M. acrita* has also been reported on tea (Chen and Chen, 1982). *M. incognita* has also been reported from Japan (Takagi, 1967). In Iran, the species of *Meloidogyne* reported to attack tea include *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla* (Nasaj Hoseini, Tehran, 2003, personal communication).

Meloidogyne species have been encountered in Bangladesh (Khan *et al.*, 2003). *M. incognita* is the only species of root knot nematode that has been identified from tea roots in nursery beds in Queensland, Australia (O'Brien, Queensland, Australia, 1988, personal communication). *Meloidogyne* spp. has also been reported to damage young tea in Argentina (S.D.P. Kricun, Argentina, 1988, personal communication).

Symptoms of damage

The species of root knot nematodes that are known to attack only young tea plants form galls on both the taproot and the feeder roots. Some root knot nematode juveniles enter the roots of mature tea bushes but fail to cause giant cells, and are apparently unable to complete the moult between the second and third juveniles (Gadd and Loos, 1946). Seedling plants in which both the taproot and the lateral roots are severely attacked suffer greater damage than the majority of vegetatively propagated tea cultivars of similar age, probably because seedling plants possess less than half the root bulk of the tea cultivars (Kerr, 1963a).

Although root knot nematodes are root feeders, the collar regions of tea seedlings have been reported to be infested occasionally with *M. incognita* in Assam, India. The females recovered from such infested locations were found to be poorly developed, although they were found to have led to the development of the characteristic galls on such affected stems (Basu, 1976).

Environmental factors affecting pathogenicity of Meloidogyne spp.

Since species of *Meloidogyne* have been encountered in almost all tea-growing regions, they seem to be well adapted to different climatic and soil conditions. In China, the optimum soil temperature for pest incidence has been reported to be 20–30°C and in soils with 20% moisture (Rong *et al.*, 1984).

Studies carried out in West Tripura, India, detected the largest population of *M. incognita* in November, when the soil was dry and rainfall was minimal in the post-monsoon season. In a 3-year-old infested field, the highest population densities were recovered 40 cm away from the base of the plant and at a depth of 40 cm (Bhat-tacharya *et al.*, 2012b).

Pathogenicity to seedling tea by *M. incognita* in India has been described by Bora and Neog (2006). Increases in inoculum level of second-stage juveniles reduced seedling height, root length and the number of lateral roots. The number of galls, egg masses and final nematode population in the soil had also increased with the increase in the inoculum level from 10 to 1000 J2/seedling, and declined at 10,000 J2/seedling.

Use of some of the herbicides has been reported to have a suppressing effect on populations of *Meloidogyne* spp. in tea fields in India (Basu and Gope, 1982; Gope and Borthakur, 1991). Since only short persistence herbicides are used in tea fields, the decline in population is probably due to the eradication of weeds that are good hosts of this species of nematode, rather than direct kill brought about by the herbicides themselves.

Alternative hosts

Species of *Meloidogyne* have a large number of alternative hosts. However, since they attack only young nursery plants, the presence of alternative hosts in mature tea fields has little influence, other than when soils from such areas are used for nursery plant propagation. On the other hand, rhizospheres of common shade trees in tea fields of Sri Lanka have been found to harbour heavy populations of nematode antagonists (Mohotti, 1998). These shade trees are not hosts of nematodes pathogenic to mature tea, but are susceptible to *Meloidogyne* spp. attacking young tea, which in turn are good hosts of many of the nematode antagonists. Therefore, these shade trees serve as reservoirs to help spread these beneficial agents into the tea fields.

Meloidogyne brevicauda

This species of root knot nematode is sometimes referred to as the tea root knot nematode, the mature tea nematode or the Indian root knot nematode, as it is the only one that attacks mature tea. It was identified by Loos in Sri Lanka in the early 1950s (Loos, 1953b), and has been recorded so far only in the tea areas of Sri Lanka, north-east India and south India. In Sri Lanka, this species has been recorded in only three plantations, all bordering the same jungle at an altitude of 1500–2000 m (Hutchinson and Vythilingam, 1963b). In south India, it has been recorded in single estates each in the Nilgiris, Wynaad and Karnataka Districts (Venkata Ram, 1963; Mehta and Somasekhar, 1998; Muraleedharan and Selvasundaram, 2001), and in north-east India it has been recorded only in Darjeeling (Mukherjee and Dasgupta, 1982).

Other than Sri Lanka and India, the other countries where this species of root knot nematode has been reported are Absheron, Azerbaijan, on saffron (*Crocus sativus*) (Kasimova and Atakishieva, 1980) and on *Morinda officinalis* (medicinal Indian mulberry), from Fujian, China (Changshang, 1984).

Symptoms of damage caused by M. brevicauda

The above-ground symptoms of attack by this species of nematode resemble those brought about by the root lesion nematode. The infested bushes are stunted as a consequence of poor recovery from successive prunes; the leaves are smaller, yellowish and dull in appearance. The roots show the characteristic presence of large galls (Fig. 16.5), many of which display pinhole pits. It is often difficult to isolate living mature females and, when found, they contain only a few eggs (Loos, 1953b).

Biology

This species was first described by Loos (1953b). Re-description of the species and additional notes on morphology were provided by Eisenback and Gnanapragasam (1992). The average size of a mature female is about five or six times that of a mature female of *M. incognita*. Despite this massive size, the females are often observed to be empty, with only a few eggs. The mean hatch per egg mass is around ten, while in the other common species this is of the order of 200–600

juveniles/egg mass (Gnanapragasam and Manuelpillai, 1981). Males are also extremely rare, and it is possible that eggs develop only following fertilization, which is likely to occur by chance, and those unfertilized fail to produce any eggs. The rest of the life history is very similar to that of the other species of *Meloidogyne*.

Early investigations in Sri Lanka revealed the presence of this species of nematode only in mature seedling tea fields (Loos, 1953b). However, subsequent studies revealed some of the tea cultivars also to be susceptible, and signs of infestation and galling became obvious in the cultivars tested after only 10 years from planting in an infested field. The most susceptible cultivar is TRI 2142, which is resistant to the root lesion nematode *P. loosi*. A low level of infestation was also observed in cultivars K 145, TC9, DT1, TRI 2024 and TRI 2025 (Gnanapragasam *et al.*, 1985). In India, to date, only seedling tea has been reported to be infested (Muraleedharan and Selvasundaram, 2001).

Environmental factors affecting parasitism

M. brevicauda needs a cool soil temperature for the build-up of populations. In studies carried out in controlled soil temperatures, successful parasitism of tea plants was observed only at 12°C, while no parasitism was found to occur at higher temperatures (Gnanapragasam, 1988). This relationship has been observed in recent field studies where increases in soil temperature in the cooler region of Sri Lanka were associated with the decreasing occurrence of *M. brevicauda* (Mohotti, 2009).

Alternative hosts

Despite intensive surveys being carried out for several years in tea areas for the possible existence of other hosts to *M. brevicauda*, to date none has been found in Sri Lanka or India. Even the weeds checked among infested tea fields have been found to be free of this species. The only alternative hosts reported so far are saffron (*C. sativus*) from Absheron, Azerbaijan (Kasimova and Atakishieva, 1980), and *M. officinalis* (medicinal Indian mulberry) from Fujian, China (Changshang, 1984).

However, since saffron as well as *M. officinalis* are not normally grown in the vicinity of tea fields, they would not pose a threat to the spread of infestation.



Fig. 16.5. Typical galling of mature tea roots caused by *Meloidogyne brevicauda*. (Photograph courtesy of K. Mohotti.)

Economic importance and population damage threshold

No information is available yet on damage threshold. Nevertheless, the intensity of damage and associated crop loss seem to be very similar to those caused by *P. loosi*. Taking into consideration the distribution of this nematode and its very limited host range (only tea, saffron and *M. officinalis*), the risk posed by this root knot nematode is small.

Radopholus similis

This species was first reported as a pest of tea in Java, Indonesia (Zimmermann, 1989). Steiner and Buhner (1933) have also reported tea to be a good host to this species of nematode. The presence of this nematode in tea in Sri Lanka was first reported in 1968, when infestations were observed in young tea fields at an elevation range of 500–1000 m (Sivapalan, 1968). Other surveys have indicated the species to be widely distributed in the tea areas (young and mature) at lower altitudes as well, up to 200 m (Gnanapragasam, 1988). In the presence of susceptible tea cultivars and under favourable climatic conditions, it is not uncommon to find *R. similis* even at very low altitudes of 50 m (Gnanapragasam, 1990). The species has also been reported from tea in China (Chen Zongmao, Hanzhou, China, 2001, personal communication), Zimbabwe and South Africa (Keetch and Buckley, 1984).

Symptoms of damage

Damage symptoms on tea are very similar to those brought about by *P. loosi*. Parasitized plants are stunted, with pale leaves (Fig. 16.6), and they go into premature flowering and fruiting, symptoms that are very characteristic of nematode damage to tea (Sivapalan, 1968). The roots of infested plants are sparse and dried up compared with the whitish succulent feeder roots of healthy plants. Although lesions have been observed on the young roots, these are very small compared with those formed by *P. loosi* on tea (Fig. 16.7) (Gnanapragasam, 1983).

Biology

As in the case of *P. loosi*, *R. similis* is attracted to the growing part of the tea roots and invades the cortical region, feeding on and destroying the cells. Being an endoparasite, in young tea most of the population is found within the feeder roots. However, when the parasitized roots are severely damaged, or when these become over-parasitized, the nematodes move into the soil in search of fresh roots. Therefore, in mature tea fields, large populations could be encountered in roots as well as in the soil in the rhizosphere of infested bushes.

Pathotypes/races

The behaviour of *R. similis* collected from the same hosts in the vicinity of tea areas from different agroecological regions varied.



Fig. 16.6. A susceptible tea cultivar (TRI 2025) infested with *Radopholus similis* (right group) compared with uninfested plants of similar age (left group). (Photograph courtesy of N.C. Gnanapragasam.)



Fig. 16.7. A tea root infested with *Radopholus similis* showing small lesions and decaying root. (Photograph courtesy of K.M. Mohotti.)

Differential host trials indicated the existence of different biological races of *R. similis* in the tea areas (Gnanapragasam *et al.*, 1991; Gnanapragasam, 1994b). This was confirmed further by molecular analysis (Hahn *et al.*, 1994).

Environmental factors

R. similis appears to be quite sensitive to cold temperatures and has a poor survival rate in tea at elevations above 1000 m. When both *P. loosi* and *R. similis* are inoculated together on to tea at high elevations, the former takes over rapidly by competitive displacement, with no trace of the latter species within a short period. However, at lower elevations, *R. similis* has been observed in the rhizosphere along with *P. loosi*. In semi-dry areas, *R. similis* also occurs concomitantly with *R. reniformis*. *R. similis* in the tea areas appears to favour uniformly distributed high rainfall. In very wet or dry soil, the population was found to decline (Gnanapragasam, 1993).

Soil type and texture were also found to have significant influence on the reproductive rate and population build-up of this pest. Detailed experiments carried out in a temperature-controlled water bath at $25 \pm 1^\circ\text{C}$ revealed a rapid build-up of populations in sandy soil, followed by gravelly or loamy soil. There was hardly any build-up in clayey soil. Damage to tea was also found to be significantly more in gravelly, sandy and loamy soil (Gnanapragasam, 1994a).

Means of dissemination and survival

The method of dissemination of *R. similis* in tea is very similar to that of *P. loosi*. However, the survival rate in host-free soil is much shorter for *R. similis*.

Some of the popular cultivars, such as TRI 2025 and TRI 2026, most favoured by small tea growers and widely planted in the mid- and lower elevations are particularly susceptible to this species of nematode and have contributed to the spread of this pest. When infested, severe damage is encountered in the nurseries and newly planted young fields, causing complete failure in establishment. The use of these tea cultivars is now being discouraged in areas prone to damage by *R. similis* (Gnanapragasam, 1983, 1995).

Alternative hosts

R. similis is polyphagous, attacking hundreds of plant species. Holdeman (1986) has reported 365 hosts. Several weeds and other plants intercropped with tea are suitable hosts to *R. similis*. Among these hosts, the most favoured ones are banana (*Musa* spp.), black pepper (*Piper nigrum*) and coconut (*Cocos nucifera*). Other common hosts include *Coffea* spp. (coffee), *Zea mays* (maize), *Saccharum officinarum* (sugarcane), *Pyrus* spp. (pear), *Persea americana* (avocado), *Ananas comosus* (pineapple), *Solanum lycopersicum* (tomato), *Anthurium andrea-num*, *Daucus carota* (carrot), *Areca catechu* (betel nut palm), *Coffea canephora* (Congo coffee tree), *Curcuma longa* (turmeric), *Dioscorea* sp. (yam), *Musa textilis* (manila hemp), *Piper betel* (betel pepper), *Zingiber officinale* (ginger), *Arachis hypogea* (groundnut) and *Solanum nigrum* (nightshade weed) (Gnanapragasam *et al.*, 1991; Gowen *et al.*, 2005; Koshy *et al.*, 2005). Due to the presence of different pathotypes of *R. similis*, populations from some of these hosts were found not to infest tea (Gnanapragasam, 1994b).

Contrary to the situation with *P. loosi*, Guatemala grass (*T. laxum*), which is often used to recondition old tea fields prior to replanting, has also been found to be a host to *R. similis* and it is currently discouraged from use in areas prone to infestation by this nematode. *E. curvula*, marigold (*Tagetes* spp.) and *V. zizanioides* appear to suppress

soil populations of *R. similis* (Gnanapragasam, 1986b, 1987b).

Economic importance and population damage threshold

In the mid- and low elevation tea areas of Sri Lanka, *R. similis* is becoming as economically important as *P. loosi* is in the high elevation tea areas. Decline among several newly planted young tea fields in the mid- and some low elevation tea areas has been associated with moderate to heavy populations of *R. similis*. Since *R. similis* is found associated with *P. loosi* in many tea areas, it is difficult to study the yield loss under field conditions. However, the results of pot experiments carried out at $25 \pm 1^\circ\text{C}$ revealed severe damage to tea brought about by a low initial population level of 28 nematodes/100 g of soil. In the field, when exposed to additional stress conditions such as drought, poor soil condition and/or attack by other pests, the damage threshold level could be even lower (Gnanapragasam and Herath, 1989).

Hemicriconemoides kanayaensis

H. kanayaensis is one of the important nematode pests of tea in Japan. It was detected originally from the roots of tea seedlings in Kanaya, Shizuoka Prefecture (Nakasono and Ichinohe, 1961), and has been detected in several other tea-planting districts in Japan (Takagi, 1969). The nematode has also been reported in Taiwan (Sivapalan, 1972; Chen *et al.*, 2007).

Symptoms of damage

This species of migratory ectoparasitic nematode feeds only on the feeder roots of tea. Continuous feeding by this nematode results in the sloughing off of the root cortex, revealing a brownish, discoloured stele (Takagi, 1969). Maximum populations are encountered at a depth of 30 cm (Kaneko and Ichinohe, 1963).

Biology

Egg laying was reported to be during May and November, with larval development occurring

in the latter half of the year, from May to November. Maximum development occurred in July. Adult males were rare and were only about 0.6% of the population (Takeshi, 1964).

A single female contains 14–15 eggs. Oviposition studies carried out in the laboratory have shown that this takes place over a period of 15–20 days during the months June/July and 35–40 days in November. The entire life cycle is reported to be completed in 100 days. The ratio of juveniles to adults was found to reach a peak in July (Kaneko and Ichinohe, 1963; Takagi, 1969).

Alternative hosts

Tea is the only reported host of *H. kanayaensis* (Takagi, 1969).

Economic importance

Large numbers of this nematode have been found to result in crop failure in tea (Takagi, 1969). An increase in nitrogenous fertilizer is reported to reduce populations of this species of nematode (Kaneko and Ichinohe, 1963; Takagi, 1969).

Other nematodes

Several other species of nematode have been recovered from the roots or root zone of tea that have not been shown to cause serious crop loss, and these are discussed below.

Rotylenchulus reniformis

The reniform nematode *R. reniformis* was first observed in tea in Indonesia (Java) in 1951, where it was found to be responsible for large-scale casualties in young tea fields (Thorne, 1961). *R. reniformis* was also reported in north-east India; however, the frequency of occurrence was very low in Darjeeling, when compared with other plant parasitic nematodes (Basu and Roy, 1975, 1976). In Sri Lanka, this species was first encountered in a tea nursery in Rakwana in 1960 and subsequently in 19 tea estates at low

and mid-elevations below 1200 m (Hutchinson and Vythilingam, 1963b). Although large numbers of nematodes were present in the root zone, no mature females could be detected. Other surveys found the tea-growing areas to be infested in the elevation range 200–900 m. Continuous soil moisture is reported to be essential for the build-up of *R. reniformis*, and reduction in rainfall below 100 mm can bring about a significant reduction in nematode numbers. Under continuous rainfall conditions, juveniles in the range of 200–370/100 g of soil were recovered from infested tea bushes. Low rainfall below 100 mm a month prior to sampling can reduce the population to 1–6 nematodes/100 g of soil, and sometimes even to non-detectable levels (Gnanapragasam *et al.*, 1987a).

Symptoms of damage

Only young tea plants were found to be infested with *R. reniformis* in Sri Lanka. The infested plants were stunted, with premature flowering and fruiting. The symptoms of damage were accentuated under poor soil conditions. Examination of the root system revealed that most of the feeder roots were clipped off due to feeding by this nematode. Although a large number of juveniles and immature females were recovered from the root zone, no mature females were found (Gnanapragasam *et al.*, 1987a).

Alternative hosts

R. reniformis has a wide range of hosts, including several common weeds encountered in tea plantations. Other perennial crops that are sometimes intercropped with tea are good hosts to this species, including pepper (*P. nigrum*), coffee (*Coffea robusta*) and young clove trees (*Syzygium aromaticum*), as well as grass cover crops, including Guatemala (*T. laxum*), which is planted in up-rooted tea fields for soil reconditioning (Gnanapragasam, 1988). Species of marigolds (*Tagetes* sp.) have also been reported to be suitable hosts for this nematode in India (Basu and Roy, 1976).

Helicotylenchus

Both *H. dihystera* and *H. erythrinae* are commonly encountered in tea soils at all elevations in Sri Lanka (Hutchinson and Vythilingam, 1963b)

and in Japan (Takagi, 1969), but no positive evidence of pathogenicity to tea has been reported from these countries. In Queensland, Australia, *H. dihystera* have been reported to affect the growth of young tea seedlings up to 12 months old. No evidence of pathogenicity has been recorded on older plants (P.C. O'Brien, Australia, 1988, personal communication). In East Africa, this species of nematode has been reported to be the most common nematode parasite in tea (Hainsworth, 1970). In Darjeeling, India, this species formed the bulk of the nematode fauna in tea soils at all altitudes. Soil samples collected from the rhizosphere of weak seedlings had significantly more numbers of nematodes than from that of healthy seedlings; however, no positive evidence of pathogenicity was demonstrated (Basu, 1967).

Paratylenchus curvatus

The pin nematode *P. curvatus* is also one of the most common and most prevalent plant parasitic nematodes encountered in the rhizosphere of tea plants at all elevations in Sri Lanka (Hutchinson and Vythilingam, 1963b), in Japan (Kaneko and Ichinohe, 1963) and in north-east India (Basu, 1967). Although large numbers of this ectoparasitic nematode are encountered in the root zone of both young and mature tea, no positive evidence of pathogenicity has yet been established.

Hoplolaimus, Rotylenchus

In north-east India, these two genera have been found in the zone of weak and stunted seedlings (Basu, 1967). These nematodes have seldom been encountered in tea soils in Sri Lanka, and in locations where they have been found, no correlation has been established between their occurrence and any setback to growth. This species has also been reported in Malawi and East Africa (Hainsworth, 1970).

Xiphinema

Large numbers of this genus have been found in the soils of tea nurseries in north-east India. They have been found to feed at the root tips of feeder roots, resulting in slight swelling of the

affected root tips. No further evidence of pathogenicity has been established with respect to this nematode (Basu, 1967). Species of *Xiphinema* have also been reported from tea fields in South Africa (Martin, 1962).

Management of Nematode Parasites in Tea

In most countries, studies on the incidence and pathogenicity of nematodes in tea have been made mostly in nurseries and young tea fields. As such, methods of control have been largely confined to the treatment of nursery soils. However, in countries such as Sri Lanka, Japan and Iran, where plant parasitic nematodes pose a serious threat to the mature tea crop, various methods have been developed to mitigate their effects on the growth and productivity of tea. In Sri Lanka for the past three decades, an effective integrated management strategy (IPM) with minimal use of pesticides has been adopted to manage tea nematodes (Gnanapragasam, 1987b). Such IPM strategies are currently being developed in other countries as well against root knot nematodes, for example, in Kenya (Kamunya *et al.*, 2008a) and Bangladesh (Mamun and Ahmed, 2011), and against root knot nematodes and *P. loosi* in Iran.

The integrated nematode management (INM) strategies adopted to manage nematodes in most of the tea-growing areas of the world focus on preventive and prophylactic methods rather than on eradicating the pests. The thorough knowledge of the biology of the specific species is integrated with various management strategies to reduce the pest below tolerable limits, and at the same time maintaining the quality of the environment and the health of the host. The various environmentally acceptable strategies include: cultural methods, physical methods, resistance and tolerance, biological control and minimal use of pesticide only when necessary.

Cultural methods

As long as the tea plant can grow vigorously and produce fresh feeder roots to compensate for those that die prematurely on account of nematode

damage, it will be able to withstand parasitism to a significant extent. Therefore, those cultural methods that enhance growth and at the same time curtail nematode soil populations help to sustain productivity at economic levels. Tea fields in which yields have declined, but not to uneconomical levels, benefit most from such practices.

Incorporation of organic matter

Besides helping in the retention of essential soil nutrients and the consequent better nutrient status of the tea plant, large inputs of organic matter, including cattle manure and well-decomposed plant residues, have been reported to suppress the populations level of *P. loosi* (Loos, 1953a; Takagi, 1969).

The incorporation of specific oil cakes, such as neem seed cake (*A. indica*), castor oil cake (*R. communis*), mahuva oil cake (*M. indica*), karanj oil cake (*Pongamia glabra*), coconut oil cake, decomposed poultry droppings, decomposed waste tea, as well as plant residues such as freshly harvested 'water hyacinth' (*Eichhornia crassipes*), help to curtail damage caused by the root lesion nematode, *P. loosi* (Gnanapragasam, 1987b, 1991, 1994b; Mohotti, 1998). Water hyacinth has been found to reduce *Meloidogyne* populations in several other crops as well (Roy, 1979; Bhargava *et al.*, 2005; Onifade and Egunjobi, 1994).

Neem (*A. indica*), and mustard oil cakes alone and in combination with nematicides were reported to suppress populations of *Meloidogyne* sp. in India and enhance growth of tea. Neem, as well as neem plus nematicide, were observed to be superior to treatments with mustard oil (Kalita and Bora, 2006). Neog and Bora (1999a) also reported neem cake and mustard cake, as well as decaffeinated tea waste, to be effective in reducing galls, egg masses and root knot nematode populations in tea nurseries in Assam, India. Mamun and Iyengar (2010) recommended neem oil cake for controlling *M. brevicauda* in India. In Bangladesh, nursery soils treated with crude plant cakes of bishkatali (*Polygonum hydropiper*), mahogany (*Swietenia mahagoni*) and neem were reported to reduce populations of pathogenic nematodes, with mahogany performing the best (Mamun *et al.*, 2014). Amendment of the soil with chopped leaves of *Glycosmis pentaphylla* reduced the reproduction of *M. incognita* and improved the growth of tea (Bhattacharya *et al.*, 2012c).

Nematode-infested plants grown in organically amended tea soils have enhanced root growth, leaf area and leaf chlorophyll content when compared with control plants treated with nematicides, and there is a decrease in tissue damage (Mohotti *et al.*, 1998, 2000a).

Soil cultivation (forking)

Soils with increasing acidity have a tendency to form a hard pan, and soil compaction impedes the rate of normal replenishment of damaged and dying feeder roots. Tea plants subjected to such conditions suffer most from nematode infestation. Regular forking of such soils helps to break the hard pan and improve soil aeration, and the consequent feeder root growth. Tea fields with a hard pan and heavily infested with the root lesion nematode *P. loosi* have recovered remarkably following such treatments (Sivapalan, 1972). An increase in soil aeration brought about by forking is also reported to increase microbial populations in the soil, thereby indirectly helping to reduce populations of nematodes (Mohotti, 1998).

Fertilizer application

The provision of balanced fertilizer mixtures influences the physiological status of the plant, which in turn influences the population dynamics of plant parasitic nematodes. An imbalanced supply of potash fertilizer (at lower proportions to increasing levels of nitrogen) was

found to enhance the pathogenicity caused by *P. loosi* in tea. The reverse effect was induced by increasing the dosage of potash fertilizer (N:K = 1:2), which also brought about a decline in the population level of this species of nematode (Fig. 16.8) (Gnanapragasam, 1982). Higher supplementation of potash was reported to reduce root knot nematodes in the tea plantations of Kenya (Kamunya *et al.*, 2008a). Increases in nitrogenous fertilizer helped reduce populations of *H. kanayaensis* (Kaneko and Ichinohe, 1963; Takagi, 1969).

The type of nitrogenous fertilizer applied to tea also influences the population dynamics of *P. loosi* in tea. The application of nitrogen in the form of urea can significantly suppress the population (Sivapalan, 1980).

Cultivation of cover crops

It is customary to plant a grass cover crop for a period of 2 years following the uprooting of old tea fields, prior to replanting with young tea. These grass species are meant to improve the physical structure of the soil, improve soil aeration and at the same time add a substantial amount of organic matter, which is provided through regular lopping of such grasses. The grass species used for such soil reconditioning include Guatemala grass (*T. laxum*) and Mana (*C. confertiflorus*), both of which are non-hosts of *P. loosi* (Visser, 1959; Hutchinson, 1962; Kerr and Vythilingam, 1966). The planting of a non-host also has the added advantage of depriving

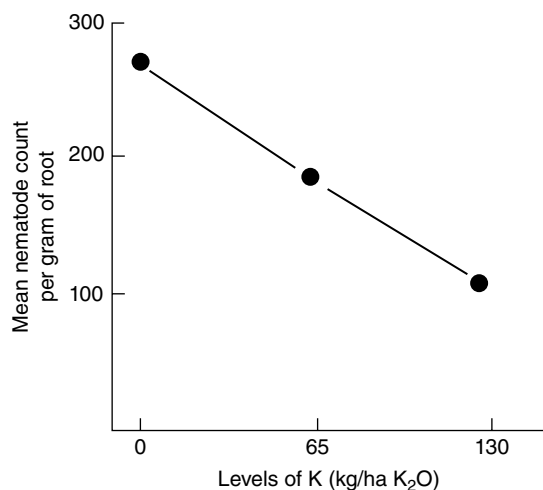


Fig. 16.8. Influence of potash fertilizer on root population of *Pratylenchus loosi* in mature tea. (Photograph courtesy of N.C. Gnanapragasam.)

the nematodes of adequate food, thus helping to bring down the population with time. Since Guatemala grass is a good host of *R. similis*, this grass is not recommended to be planted in areas infested with this species of nematode (Gnanapragasam, 1995). In Bangladesh, Guatemala grass *Tripsacum laxum* and *Citronella* are being used for field rehabilitation, as these have been found to be non-hosts of nematodes infesting tea in this country (Mamun *et al.*, 2011).

Planting of antagonistic crops

In the past, vacant areas among nematode-infested old tea fields were sometimes planted to marigolds (*Tagetes erecta* and *Tagetes patula*) to help to reduce populations of *P. loosi*, prior to infilling such areas with young tea. Since marigold competes for soil moisture and nutrients, this practice was discouraged in young tea fields and such planting was confined to the older tea areas only (Hutchinson, 1964; Hainsworth, 1970). The nematode-suppressing activity of marigold is most effective during its phase of vegetative growth and prior to flowering (Hutchinson, 1961; Sivapalan, 1972), and thus it is not practical to plant marigold in tea fields. It is now recommended for planting along the boundaries of nurseries to deter nematode migration and infestation. Marigold *Tagetes minuta* was observed to reduce populations of *Meloidogyne* in tea fields of Kenya, but retarded tea growth and thus was found not to be suitable for intercropping with tea (Kamunya *et al.*, 2008a). The radish cultivars Defender, Colonel and Boss, have also been found to be very effective in suppressing populations of *P. loosi* and *R. similis* in tea soils (Mohotti, 2009).

The planting of *E. curvula*, which is planted mainly to prevent soil erosion in steep sections and in vacant areas in tea fields, has been found to suppress populations of *P. loosi* (Gnanapragasam, 1981) and *R. similis* (Gnanapragasam, 1986b). This grass has also been reported to suppress populations of *Meloidogyne* sp. in tea fields in Malawi (Anon., 1960).

The other trap crops that are recommended to be planted in the vacant areas of tea fields include *A. pintoi*, *T. diversifolia* (wild sunflower), *Vinca rosea* (periwinkle) and *V. zizanioides* (vetiver) (Gnanapragasam, 1995, 1997).

Plant species such as *A. vasica*, *S. javanica*, *I. teysmannii*, *E. inuliformes*, *C. calothyrsus*, *C. anagyroides* and *Lantana camara* also seem to possess nematicidal properties, as these have been found to reduce drastically populations of both *P. loosi* and *R. similis*. These crops do not compete for moisture and nutrients and are suitable to be grown as hedgerow planting in steep areas. Apart from reducing populations of nematodes, these plant species also help to add adequate mulch to help build up the organic bulk of soil (Gnanapragasam, 1997; Gnanapragasam and Sivapalan, 2001, 2004).

Irrigation

Nursery plants that are irrigated with water collected from ravines that course through infested sections of tea plantations have been found contaminated with nematodes (Gnanapragasam and Jebamalai, 1982). In order to circumvent this danger in areas prone to nematode infestation, it is a recommended practice to sediment irrigation water in specially built sedimentation tanks for 48 h. In areas where it is not practical to build sedimentation tanks, Mohotti (2010) has recommended the treatment of irrigation water with bleaching powder (at 100 g/10 l of water), which is a cost-effective alternative treatment. Such treatment can also be used to treat contaminated soil in nurseries.

Resting of tea fields

Tea fields are pruned regularly once every 2–4 years, depending on the ambient temperature of the locality. This is a drastic operation, the recovery from which is dependent on the physiological condition of the pruned tea bush. When the tea field is subject to various forms of stress, including nematode parasitism, the affected sections of the tea fields recover poorly, mainly on account of the low carbohydrate reserves. In order to overcome this, such fields are rested prior to pruning for periods ranging from 6 to 8 weeks.

Replanting old tea fields

Tea fields that are uneconomical for further retention are uprooted and replanted to selected tea cultivars with specific virtues. If such fields are known to be infested with nematodes, the uprooting of the old tea has to be carried out in

such a manner as to ensure the extraction of as many residual roots as is possible. Large root fragments left in the soil harbour nematodes in the periphery of the lesions and such populations are known to remain viable for as long as 2–3 years (Hutchinson, 1960, 1964). Large-scale failures in newly planted tea areas have been traced to reinfestation from residual populations remaining in old roots (Sivapalan, 1967b). It is, therefore, extremely necessary to ensure a thorough cleaning up of all residual roots following uprooting.

Reinfestation could also occur rapidly through the movement of soil from an infested area higher up on the hill slopes or the crest of the hill. Therefore, when replanting is undertaken in areas prone to nematode infestation, the uprooting of tea should commence from the top of the hill downwards, and not vice versa (Gnanapragasam, 1985a).

Physical control

The only physical control method adopted for controlling nematodes in tea is in nurseries. Nursery soil used for propagating young tea plants in India is sometimes heated by spreading the soil on galvanized sheets to temperatures ranging from 60 to 62°C for 5 min (Rao, 1976; Basu, 1978; Muraleedharan and Selvasundaram, 2001) or steam sterilization at 15 lb psi for half an hour (Neog and Bora, 1999b; Muraleedharan and Selvasundharan, 2001). This method of nursery soil treatment is not practical for large nurseries and is not recommended in Sri Lanka, as this could damage the soil tilth. It is also more suitable for controlling root knot nematodes.

Resistance and tolerance

The use of resistant/tolerant cultivars is the most economical and environmentally friendly component of a nematode management programme. Tea cultivars have been assessed to have varying degrees of natural tolerance and resistance to different species of parasitic nematodes in Sri Lanka (Loos, 1953a; Hutchinson, 1960b). Large numbers of tea cultivars have been screened in Sri Lanka for resistance and tolerance to the root

lesion nematode *P. loosi* and *R. similis*, and several cultivars have been recommended for planting in nematode-infested areas in the different agroclimatic regions of Sri Lanka (Anon., 2002).

The screening of tea cultivars has been carried out for root knot nematodes in Kenya (Kamunya *et al.*, 2008b, 2011; Orisajo, 2012) and in China (WeiXi *et al.*, 2011).

During recent years, the breakdown of resistance has been observed among some of the cultivars earlier assessed to be resistant/tolerant, and such breakdown of tolerance/resistance was found to have been brought about by the age of the plants and adverse environmental factors (Gnanapragasam, 2002b; and unpublished data). Some of the high-quality popular cultivars, such as DT 1, earlier assessed to be tolerant to *P. loosi* are presently found to be very susceptible at the initial stages of growth (Gnanapragasam, unpublished data). Therefore, continuous monitoring of such chosen cultivars becomes essential to assess such breakdown of tolerance/resistance.

Grafting

In Sri Lanka, some of the high-yielding tea cultivars that are renowned for good quality were found to be susceptible to *P. loosi* and/or *R. similis*, and therefore not suitable for planting in tea areas. Grafting a nematode-susceptible scion on to a nematode-tolerant/-resistant rootstock has been found to induce resistance/tolerance in such combinations, making it possible to use high-yielding but nematode-susceptible cultivars in the field (Gnanapragasam, 1992). When choosing such graft combinations, only those cultivars that are compatible with each other and suitable for growing in the particular agroclimatic zone are selected.

Chemical control

In a perennial crop that is grown for as long as 50–60 years, chemical control generally proves to be uneconomical, since such costly treatments have to be repeated periodically to sustain populations below economic thresholds. This form of treatment is thus confined to eradicating nematodes from nursery soils and in reducing soil populations in the field at the time of planting the young tea.

Chemical control in nurseries

Infested nursery plants are an important source of spread of nematode infestation to fields that may hitherto have been free of infestation (Fig. 16.9). In areas prone to such infestation, it is a routine practice to treat all nursery soils chemically. Nursery soils in the past were treated with fumigants such as methyl bromide, DD soil fumigant, Nemagon 75% E.C. and Dowfume W 85% (Kerr, 1963b; Akbar and Ali, 1965a,b; Sivapalan, 1969; Nara *et al.*, 1973). Since the early 1980s, as an alternative to methyl bromide, Dazomet (Basamid 98% G) was recommended in Sri Lanka (Sivapalan *et al.*, 1980). Basamid 98% G has also been used effectively to control soil nematodes in Bangladesh tea nurseries (Huq *et al.*, 1990). Currently, metam sodium is also being used in Sri Lanka (Anon., 2012). Soil solarization has also been attempted in Sri Lanka (Vitarana, 2001). However, since large volumes of soil are used in tea plantations each year for propagating nursery plants, and

owing to low sunshine hours in tea-growing regions, solarization is not practical, and the results of such trials have been inconclusive. Studies on the biofumigation of tea soils using *Brassica* plants (raddish and cabbage) initiated by Mohotti (2010) are reported to be promising, and may prove to be an alternative technology in the future.

Following such chemical treatment in the nursery, the cuttings or seeds are planted after an appropriate interval as specified for the respective chemical. In certain countries such as India and Bangladesh, granular nematicides are added to soils bearing young nursery plants (Rao, 1974, 1976; Basu, 1979; Basu and Gope, 1985; Huq *et al.*, 1990). In Sri Lanka, such treatment of nematode-contaminated nursery plants with granular nematicides is recommended only under very special circumstances; the cultivar involved has to be a tolerant one and infested with only a light population of nematodes and not exhibiting any damage symptoms. Treatment is also limited to instances



Fig. 16.9. Young tea plants in a nursery heavily infested with *Pratylenchus loosi*. (Photograph courtesy of N.C. Gnanapragasam.)

when these treated plants are to be used only as infillings in fields already having a history of nematode infestation (Gnanapragasam *et al.*, 1987b). In addition to ensuring that tea plants are propagated in nematode-free soil, basic nursery hygiene is also maintained to prevent further contamination (Gnanapragasam, 1989).

Chemical control at planting

Despite soil rehabilitation and minimizing residual populations in the soil, and further confining the replanting to those cultivars that have proven tolerance or resistance to nematode infestation, chemical treatment of planting holes at planting time is practised routinely in Sri Lanka. This practice is carried out as an additional measure of insurance against a possible setback to establishing young plants. Since the removal of fenamiphos and carbofuran from the market, new environmentally friendly, short persistence nematicides and biopesticides are being screened as prophylactic treatments in Sri Lanka. Organophosphate nematicides are used in Iran to control nematodes in tea fields (Maafi and Moghadam, 2001).

Chemical control in mature tea

Routine chemical treatment of mature tea is an uneconomical exercise. Nevertheless, each time the tea is pruned, a significant amount of feeder roots decay, and at the time of recovery from pruning, there is a significant growth of new feeder roots that are susceptible to rapid reinfestation, and this has a significant deleterious effect on the rate of recovery from pruning. Therefore, in Sri Lanka, for fields that still have a high yield potential but are subject to moderate to heavy nematode infestation, a single application of nematicide (fenamiphos or carbofuran 5% G) at 7 g/plant, mixed along with the first application of fertilizer following pruning was recommended (Gnanapragasam, 1987b). With the removal of these two chemicals from the market, environmentally friendly, plant-derived chemicals (botanicals) are being screened to control nematode pests in mature tea. Derivatives of neem have been found to be effective in controlling *P. loosi*. In Taiwan, two derivatives from cinnamon oil (NPP_D and NPP-H) have been found to be effective in controlling *M. incognita* (Yen

et al., 2008). Leaf extracts of indigenous medicinal plants *G. pentaphylla* and *Holarrhena antidysenterica* were also observed to have nematicidal properties against *Meloidogyne* attacking tea (Bhattacharya *et al.*, 2012c).

Biological control

Until recently, very little information has been available with regard to the control of plant parasitic nematodes in tea by biological agents.

In the past, a sporozoan endoparasite was recorded occasionally from *P. loosi*, but its significance in controlling this pest was not confirmed. The presence of predatory nematodes in tea soils of Sri Lanka was also reported but, since they were not found in large numbers, no attempt was made to investigate their efficiency in controlling plant parasitic nematodes of tea (Gadd and Loos, 1946). The use of fungi in the control of nematodes has been reported by Barua (1983) in north-east India. Kaneko and Ichinohe (1963) have reported a phycomycete fungus to be responsible for as much as 30% reduction in the population of adult females of *H. kanayaensis*.

Although compost and soil amendments have been included in the integrated management programme in Sri Lanka for several years with the intention of helping to increase the natural predators and parasites of nematodes pathogenic to tea, no investigation had been carried out to isolate and identify the predators and parasites involved in such suppression of nematodes.

Several microbial antagonists of plant pathogenic nematodes have been found to be present in tea soils (Table 16.2.). Mohotti (1998) reported the frequency of occurrence of *Pasteuria penetrans* to be relatively low compared with other species in the Sri Lankan soils. The most microbial antagonists encountered are the nematode-trapping fungi (*Arthrobotrys musiformis*, *Arthrobotrys oligospora*, *Arthrobotrys* sp., *Dactylella* sp., *Monacrosporium* sp.), *Fusarium* sp., *Purpureocillium* sp. and *Trichoderma* sp. *P. penetrans* has also been reported in the tea soils of Iran (Mohotti *et al.*, 1996). The incidence of *P. penetrans* and nematophagous fungi is reported to be high in tea soils when compared with other surveyed agricultural lands of Sri Lanka (Mohotti *et al.*, 1996; Mohotti, 1998). As is to be expected, microbial antagonists of pathogenic

Table 16.2. Records of naturally occurring nematode antagonists in tea soils.

Biocontrol group	Organism	Country of report	Reference
<i>Bacteria</i>	<i>Pasteuria penetrans</i> group	Iran	Barooti (1989); Mohotti (1998)
	<i>Bacillus</i> sp.	Sri Lanka	
	<i>Bacillus subtilis</i>	India	Pandey <i>et al.</i> (2001)
	<i>Pseudomonas fluorescens</i>	Iran	Rahanandeh, <i>et al.</i> (2012)
Nematophagous fungi	<i>Fusarium</i> sp.	Iran	Rahanandeh <i>et al.</i> (2013)
		Japan	Kaneko and Ichinohe (1963); Mohotti (1998)
	<i>Purpureocillium</i> sp.	Japan	Kaneko and Ichinohe (1963); Mohotti (1998)
		Sri Lanka	
	<i>Trichoderma harzianum</i>	India	CAB International (n.d.)
	<i>Trichoderma koningii</i>	India	Pandey <i>et al.</i> (2001)
	<i>Trichoderma</i> sp.	Sri Lanka	Mohotti (1998)
	<i>Trichoderma viride</i>	India	CAB International (n.d.)
	<i>Verticillium</i> sp.	Sri Lanka	CAB International (n.d.); Mohotti (1998)
Nematode-trapping fungi	<i>Arthrobotrys musiformis</i>	Sri Lanka	Mohotti (1998)
	<i>Arthrobotrys oligospora</i>	Sri Lanka	Mohotti (1998)
	<i>Arthrobotrys robusta</i>	Sri Lanka	CAB International (n.d.)
	<i>Arthrobotrys</i> sp.	Sri Lanka	Mohotti (1998)
	<i>Dactylella</i> sp.	Sri Lanka	Mohotti (1998)
	<i>Monacrosporium</i> sp.	Sri Lanka	Mohotti (1998)
Microarthropods	Tardigrades (water bears)	Sri Lanka	Hutchinson and Streu (1960)
	Collembolans (springtails)	Sri Lanka	Mohotti (2002)
	Diplurans	India	Jauhari and Madan (2002)
	Mites (Acari)	Sri Lanka	Gadd and Loos (1946); Mohotti (2002)
Nematodes	Myriapods	Sri Lanka	Mohotti (2002)
	<i>Mononchus</i> sp.,	Sri Lanka	Gadd and Loos (1946)
	<i>Diplogaster</i> sp. and		
	<i>Dorylaimus</i> sp.		
Miscellaneous	VAM (vesicular arbuscular mycorrhiza)	Sri Lanka, Taiwan	Balasuriya <i>et al.</i> (1991); Chang and Young (1992)
	Protozoans	Sri Lanka	Gadd and Loos (1946)

nematodes are activated, conserved and enhanced in soils incorporated with organic amendments (Mohotti *et al.*, 2000b).

The host cuticle of *P. loosi*, *R. similis*, *M. brevicauda* and *Pratylenchus* sp. can be encumbered with the endospores of *P. penetrans* (Mohotti, 1998). In *R. similis*, the endospores enter the pseudocoelom.

Microbial antagonists such as strains of *Bacillus subtilis* (Rahanandeh *et al.*, 2012) and *Pseudomonas fluorescens* (Rahanandeh *et al.*, 2013) isolated from the tea soils in Iran are also being harnessed as biocontrol agents to control *P. loosi*. Introducing biological control methods in the integrated management system in tea

fields may be effective in controlling plant parasitic nematodes.

Nematode management in organic tea culture/gardens

Recently, there has been a high demand in certain countries for high-quality, organically grown tea, which fetches a significantly higher price than that grown in conventional systems. In organic farming, the plants are grown in an environment free of chemicals. Such an integrated farming system causes the least disruption to the environment, helps to improve soil

fertility, helps to enhance microbial activity and can evolve a sustainable farming system. In Sri Lanka, nematode management under such a system is made possible by harnessing eco-friendly alternative methods of control without the use of any nematicides. Some of these recommended practices include: (i) use of non-hosts; (ii) use of resistant/tolerant cultivars of tea; (iii) planting of nematode antagonistic plants; (iv) use of botanicals; (v) use of soil amendments; and (vi) use of naturally occurring biological control agents (Mohotti, 1998, 2001; Mohotti *et al.*, 1999; Gnanapragasam and Sivapalan, 2001).

Summary of management methods

Nematode management should commence at the nursery stage itself to ensure that only healthy, vigorously growing, nematode-free plants are transferred to the field. Nursery plants should be grown in nematode-free soil; proper precautions should be taken to adopt adequate hygienic measures to prevent plants becoming contaminated from adjoining fields; and transportation of nursery plants from one plantation to the other should be discouraged.

Although nematodes cannot be eradicated in a field, it is essential to reduce them below the economic damage threshold to help to avert a reduction in crop productivity. Any one of the management strategies by themselves may not be adequate to reduce the population below the economic damage threshold in the field planted to young and mature tea. In Sri Lanka, the management strategy during the past two decades has, therefore, been an integration of the appropriate methods of control most suited for a given environment.

The most useful resources for nematode management in tea fields are:

- limited use of environmentally friendly chemicals with short soil persistence;
- planting of nematode-tolerant and -resistant cultivars;
- planting of healthy and nematode-free planting materials;
- proper soil management to maintain soil pH within the range of 4.5–5.0;
- using potash-enriched fertilizer mixtures;
- enriching the soil with various organic matter;
- cultivation of soils by regular forking;
- use of antagonistic crops and botanicals;
- use of biological control agents;
- adhering to good agricultural practices (GAPs) to minimize host plant stress.

Method of diagnosis

As is the case with the other crops, the above-ground damage symptoms on tea brought about by nematodes are often confused with similar symptoms induced by other factors that tend to restrict root growth. The removal of suspect bushes indicates (if infested) an almost complete absence of feeder roots, or if feeder roots are present, they would be few and appear dead or dried up. In the case of lesion nematodes, when the bark is peeled lightly, dead brown areas (lesions) can be observed (see Fig. 16.3), whereas those infested with root knot nematodes will show typical galls and knots. In the case of the latter, inspection of perineal patterns or molecular tools will indicate the specific species involved. Positive diagnosis of migratory nematodes such as lesion nematodes is made by sampling both soil and roots from affected sections and extracting the nematodes by a modified Baermann funnel technique (see Chapter 4, this volume). The efficiency of recovery by the Baermann funnel technique is significantly improved by the addition of small amounts of tea root saponins (1–10 ppm). Further addition beyond 10 ppm suppresses recovery (Sivapalan *et al.*, 1979). Storage of soil samples as well as sample size of roots has an effect on the efficiency of recovery (Gnanapragasam and Sivapalan, 1991).

Soil sampling

Sampling of tea soils should be carried out routinely in all suspect areas for all species of nematodes that are pathogenic to tea. In Sri Lanka, sampling for *P. loosi* and *R. similis* are usually carried out when the soil is adequately moist at a depth of 15–25 cm and at a distance of 15 cm from the base of the tea plant. Several samples are collected from a given field, with approximately 25–30 randomly collected samples per 2 ha. If only a section of a field is found to be showing decline symptoms, samples are collected from such specific locations. When collecting

the samples, it is necessary to sample the weak as well as the moderately healthy plants, since very weak plants carry only a small population during growth decline. It is essential to include a few feeder roots as well as the small root fragments that come with the soil.

However, for a proper sampling of *H. kanayanaensis*, sampling should be done deeper, at a depth of 30 cm (Kaneko and Ichinohe, 1963; Takagi, 1969). Besides recovering the above species of plant parasitic nematodes, several other species of tea nematodes could also be recovered from these samples, and thus a good knowledge of nematode identification is required.

Root sampling

When a newly planted young tea area is to be sampled for nematode infestation (young tea fields less than 5 years old), it is necessary to collect feeder root samples as well. A good representative field sample will contain between 25–30 random subsamples per hectare. If only a section of the field appears to show decline symptoms, the collection of samples is confined only to the specific suspect area. A few grams of feeder roots are collected from the rhizosphere of these respective points of sampling and bulked together to form a composite sample.

Detection of *Meloidogyne* spp.

Meloidogyne species can be detected by examining the roots of the suspect tea bushes and checking for the presence of characteristic swellings and/or galls, as well as for the presence of females and egg masses clinging on to the outside of the root. Infestation with *M. brevicauda* can be distinguished easily from the other *Meloidogyne* species by the size of the female, which is significantly larger than the other common species of root knot nematodes encountered in tea fields.

Conclusions and Future Prospects

Although pathogenicity to tea caused by nematodes has been known for several years, the damage caused by this pest is not yet taken seriously by many tea-growing countries.

Therefore, it is necessary to create a greater awareness in all tea-growing countries to

recognize this problem, and greater emphasis must be placed on carrying out extensive surveys to identify the presence of different species of pathogenic nematodes and study their interaction with other environmental factors to help quantify crop losses brought about by this pest in different situations. It is only when such visual symptoms of damage are correlated with economic crop loss that the severity of nematode damage becomes more apparent to those who are not aware of the problem.

Molecular and biochemical studies should also be actively pursued to help to identify different races/pathotypes and study their interactions and pathogenicity on different hosts, as well as their interactions under different environments. These studies would further strengthen the integrated management programme and help in providing recommendations suited for specific locations.

Recently, some of the tea cultivars assessed to be resistant to pathogenic nematodes have been found to succumb to infestation as a result of the breakdown of resistance/tolerance with the age of the plants and exposure to adverse environmental factors. Since market requirements for tea pose restrictions with genetically modified organisms, genetic modifications of the tea plant have no future in the management of tea nematodes. Therefore, greater effort should be made to continue to screen cultivars for natural tolerance and susceptibility to this pest under different environmental conditions.

Recent developments in genetic mapping to identify markers linked to resistance traits of tea cultivars and plant parasitic nematodes should be expanded to all nematodes of economic importance to tea (including specific nematode isolates and/or races).

Harnessing DNA applications to detect populations of nematodes in soil would serve as an important tool for decision making with regard to the need for nursery soil sterilization and determination of soil treatments for all nematodes.

It is also necessary to strengthen studies on biological control further, so that potential formulations of microbial antagonists could be used in nematode management. This will be especially useful in organic farming areas.

Interactions with other scientists in the field of pathology and entomology should also be strengthened to study disease complexes

brought about by a combination of pest incidence, such as nematode and insect or nematode and fungi, etc.

Studies should also be carried out to control these pests at the physiological level using pheromones and specific metabolic disruptors.

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17 Nematode Parasites of Bananas and Plantains*

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Bananas (*Musa*) thrive in the lowland tropical regions where rainfall exceeds 1250 mm/year, with temperatures above 15°C, and are produced across tropical regions. Significant areas of production exist outside these climatic zones, such as in the East African highlands, several subtropical countries and in warmer localities beyond the 30° latitudes (Stover and Simmonds, 1987; Robinson, 1996). Bananas originate in South-east Asia and the western Pacific islands, where several wild, seed-bearing *Musa* spp. still exist in the natural vegetation. There is no firm botanical distinction between the varieties of banana, and they are best classified by dividing the many different types into those that are sweet and eaten as a dessert fruit and those that can be eaten only after cooking, or are used to produce juice, mostly for fermenting into a nutritious type of beer. In many countries, the cooking bananas are known as plantains, but the term is sometimes used ambiguously, as many cooking bananas are not plantains. All edible bananas are sterile, and are propagated vegetatively. Of the great number of recognized clones (Stover and Simmonds, 1987; Daniells *et al.*, 2001), some are derived from *Musa acuminata* and others from natural hybridizations of *M. acuminata* and *Musa balbisiana*. Currently accepted

nomenclature of clones indicates ploidy and genomic origin, with A for *acuminata* and B for *balbisiana*.

Of the most commonly cultivated clones, the dessert bananas that are produced for the international trade (the Cavendish clones) are triploid *Musa* AAA, as is the once popular Gros Michel; Silk, Mysore, Pome and Prata are *Musa* AAB; Sucrier and Pisang Mas, *Musa* AA; and Ney Poovan, *Musa* AB. The Cavendish cultivars are all minor variants of one genotype; a convenience for the major export trading companies, but a major risk from a crop protection perspective. There is no other major fruit or vegetable crop that depends solely on one cultivar. The entire infrastructure, including packaging, refrigeration, shipping and marketing, is geared solely to the Cavendish cultivars.

Plantains (*Musa* AAB), often referred to as cooking banana, are in fact quite different to the East African highland cooking bananas (Matooke) (*Musa* AAA), which in turn are different from Mchare cooking banana (*Musa* AA), and indeed to Bluggoe and Pisang Awak (*Musa* ABB). Plantains and other cooking bananas are often overlooked as important due to being overshadowed on the global arena by the dessert export industry. However, they are extremely

*A revision of the chapter by S.R. Gowen, P. Quénéhervé and R. Fogain in the second edition.

important as a food crop, on which millions of people rely as a staple food source. The East African highland bananas are a staple food for over 30 million people in eastern and Central Africa alone. Plantains and cooking bananas are eaten in many parts of the world after being roasted, steamed, stewed, processed or brewed, depending on the specific banana type, region and/or cultural preference. International trade in dessert and cooking bananas amounts to 20 Mt, and estimated world production is 114 Mt (FAO, 2014).

The diversity in diploid, triploid and tetraploid clones has been widened through synthetic hybridizations, some of which are now in cultivation. Besides regional field collections, over 1500 accessions of *Musa* are stored in tissue culture in the International Musa Transit Center at the Katholieke Universiteit, Leuven, Belgium, in cooperation with Bioversity International. This collection is held in trust under the auspices of the Food and Agriculture Organization (FAO) (Bioversity International, 2017).

Bananas grown by smallholders are grown mostly for local consumption and household revenue in mixed cropping systems with little management. These systems are characterized by a general lack of knowledge of the presence of nematodes as a yield-limiting factor. The yield of cooking bananas in East Africa is around 10 t/ha compared to 60 t/ha in other parts of the world. The impact of nematodes and diseases on yield in these regions is immense.

Bananas are herbaceous perennials with short, underground rhizomes from which an adventitious root system develops, with up to 300 first-order cord roots. These may grow up to 3 m laterally from the rhizome (corm) and are mostly in the upper 40 cm of soil (Araya *et al.*, 1998). Fewer roots grow vertically or deeper, although rooting density and distribution are influenced by the texture and depth of the topsoil (Irrazary *et al.*, 1981; Weckx, 1982). There are genotypic differences in root architecture (Gowen, 1993; Blomme *et al.*, 2003), a feature that breeders could exploit. New roots are produced continuously until flowering, which may occur from 7 to 9 months after planting a new crop of the commercial AAA cultivars. The duration of the vegetative phase may be considerably longer if climatic or soil conditions are less favourable, and over 1–2 years in cooler upland regions. After

flowering, natural senescence of the root system is hastened by root pathogens. The increasing root growth of the daughter plant (ratoon crop) may be of benefit during this critical phase by providing additional anchorage to the mother plant, and also as a supplementary source of nutrients for the maturing fruit (Lavigne, 1987).

Enset (*Ensete ventricosum*) is a plant belonging to the family Musaceae. In the farming communities of Ethiopia, different parts of the plant are consumed as food, especially the corm, which is used as a staple source of food, to prepare flour for bread, as well as a fermented beverage. The younger parts of the pseudostem are eaten as a vegetable. Enset leaves are used for roofing and other household purposes.

Banana propagation techniques

Suckers

Traditionally, propagation involves the removal of lateral shoots (suckers) that proliferate around the parent plant, which are then planted directly into the field, mostly without any treatment. The larger the sucker size, the greater the germination success. The disadvantage of this technique is that the suckers harbour pests and diseases arising from the field, which serves as the greatest single source of root-borne pest and disease dissemination, especially nematodes (Fig. 17.1). This form of planting is now restricted mostly to smallholder and subsistence farmers or to farmers who do not have nematodes nor black weevil problems.

Tissue culture plants

The development of the meristem tissue culture technique, which generates an abundance of new shoots in sterilized growing media (Israeli *et al.*, 1995) has revolutionized banana propagation. The opportunity for preventing the spread of numerous pests and diseases is one of several advantages of using this system. Most commercial growers have adopted the use of *in vitro* plants as a norm whenever replanting becomes necessary (Fig. 17.2). Commercial tissue culture laboratories, capable of producing many millions of plants, are now established throughout the world. Tissue culture plants are also being promoted



Fig. 17.1. Banana suckers for use as planting material by smallholder growers in Tanzania (a) and Uganda (b), and enset in Ethiopia (c), infected with plant parasitic nematodes and other organisms. (Photographs courtesy of D. Coyne and P. van Asten.)

for smallholders in non-exporting countries (Fig. 17.3) (e.g. Dubois *et al.*, 2013). In many situations, access to large numbers of good quality plants can be a problem for farmers, but a key issue is awareness and understanding of the benefits of using this more costly planting material. With increasing knowledge of the benefits, and with improved nursery networks to accommodate and support farmer access, farmers will be more prepared to invest in such better quality planting material. A problem persists, however, on both commercial and non-commercial banana plantations, in that plantlets are often planted into infested soil. Still, the benefits of using such healthy planting material outweigh the disadvantages (Kabunga, 2011).

Macropropagation plants

Various techniques are available that can substitute for tissue culture plants when access is not possible, especially for use with smallholders

(Tenkouano *et al.*, 2006). Macropropagation is a key improvised method for producing and supplying healthy planting material (Lefranc *et al.*, 2010). The technique is based on the use of corms, ideally sourced from healthy plants. The corm needs to be 'cleaned' by removing roots, trimming necrotic areas and then disinfected with hot/boiling water. The corm can then be incubated, preferably after splitting into two halves, in a nematode- and disease-free medium, such as sawdust, using a simply constructed frame covered in polythene sheeting, which maintains high humidity. Plantlets sprout from the corm surfaces, which can be removed once they have reached a suitable size, transplanted into pots and weaned before use or sale to farmers (Fig. 17.3).

Cultivation techniques

In many countries, banana and plantain rapidly become unproductive for reasons



Fig. 17.2. Large-scale commercial tissue culture derived micropropagated banana plantlets in Costa Rica (a, b and c). (Photographs courtesy of A. zum Felde.)

related to the soil structure, fertility, drainage and the severity of pathogens and nematodes, so that frequent replanting is necessary (Stover and Simmonds, 1987). The intensity of

inputs and management for the different farming systems are quite varied and depend on the market or use for which the fruit is destined.



Fig. 17.3. Simple structure for macropropagation of banana plantlets (a); macropropagated banana plants being weaned in pots before transfer to the hardening nursery for transplanting to the field (b). (Photographs courtesy of D. Coyne.)

Banana for export

All dessert fruit and some cooking bananas grown for the international export trade are managed intensively to ensure high yields of fruit of the correct size, free of skin blemishes and postharvest diseases. Such fruit is usually produced in pure stands at densities maintained at 1700–2000 plants/ha. Irrigation is applied where rainfall is inadequate; a minimum of 100 mm of rain/month is considered ideal.

Non-export banana

Bananas are a valuable component in mixed farming systems, providing continuity of food, income and employment throughout the year. Mixed intercropping in the same field is common in smallholder situations. Fruit can be harvested close to maturity, and little attention is given to fruit size and skin blemishes. Field operations may be conducted if necessary, to prevent crop loss. Nematodes are often a serious problem in that rotation with non-host crops before replanting is never followed, and nematicides, a standard tool in commercial production, are unavailable or farmers are unaware of their benefit. Although farmers are well aware of toppling problems, they are unaware that nematodes are the cause, and consequently are unaware of how to deal with the problem. Often, farmers attribute weevils to toppling damage. Under subsistence production systems, replanting nematode-infected suckers is commonplace.

Banana as a subsistence crop

Across the tropics, the vast majority of households have banana growing close to the homestead. These stands are habitually mulched with household waste, plant debris and animal manure, unlike plantings at a distance from the home. These plants are usually propped when maturing fruits are present (Fig. 17.4).

Nematodes of Banana, Plantain and Enset

The most widespread and important plant parasitic nematodes are *Radopholus similis*, some species of *Pratylenchus* and *Helicotylenchus multicinctus*. As for most tropical crops, nematode parasitism in banana roots is characterized by simultaneous infections by several species. It is also very common to find some sedentary parasites, such as *Meloidogyne* spp. and *Rotylenchulus reniformis*. In addition to these five major nematodes, there are numerous reports of other species from *Musa* spp. throughout the world, although they are not normally considered serious pests.

Depending on the nematode species, symptoms will vary from the most severe, such as toppling, to the less obvious, such as prolonging of the vegetative cycle. In situations where toppling is common, crop loss can be extreme, because the immature fruit on a fallen plant generally has no value.

Nematodes parasitizing enset have received little attention. Peregrine and Bridge (1992) reported *Pratylenchus goodeyi* to be a major problem on enset in Ethiopia. In a survey of 25 enset sites in seven agroecological zones of Ethiopia (Bogale *et al.*, 2004), the most common nematode species detected were *P. goodeyi*, *Aphelenchoides ensete* and species of *Meloidogyne*. *A. ensete* was isolated from young plants exhibiting severe black, streak-like symptoms on the leaves. The nematode appears to affect most enset clones, infecting the leaf tissue (Quimio and Tessera, 1996). Severe infections can cause the premature death of leaves and can severely damage enset suckers and seedlings (Coyne and Kidane, 2018). The removal of infected leaves reduces its spread.

Radopholus similis

The disease of banana caused by *R. similis* is known throughout the world by different names, the most common being 'black head toppling disease' and 'toppling disease' (Fig. 17.5a and b). The burrowing nematode, *R. similis*, first observed by Cobb in necrotic tissue of *Musa* sp. roots sent to him from Fiji in 1891, is now found in almost all tropical and subtropical banana- and plantain-growing regions, except Israel, the Canary Islands, the Cape Verde Islands, Cyprus, Crete, Mauritius and Taiwan (Fig. 17.6). It is also absent from some of the important areas of production in the highlands of East and Central Africa, where temperatures at the higher altitudes



Fig. 17.4. Subsistence farming system with varietal mixture of banana types in East African highlands (a), compared with double-row intensive monoculture of Cavendish banana in a commercial plantation in Taiwan (b). (Photographs courtesy of P. van Asten and A. zum Felde.)

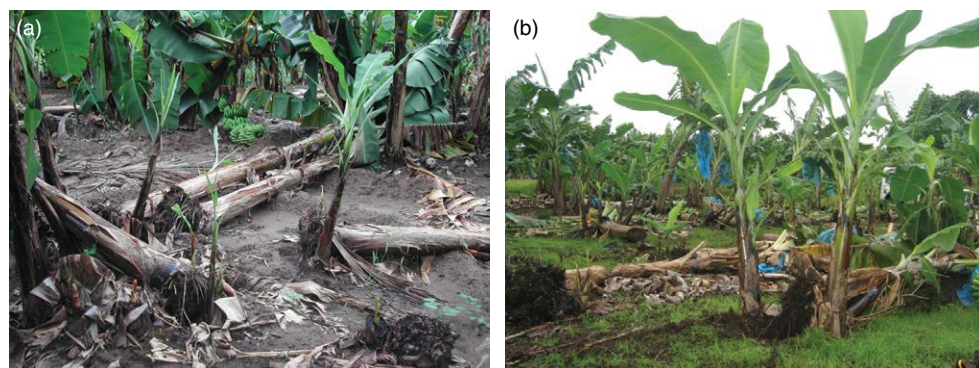


Fig. 17.5. Toppled bananas due to nematode infection and reduced anchorage of the banana root system in Kenya (a) and due to *Radopholus similis* in Martinique (b). (Photographs courtesy of D. Coyne and P. Quénehervé.)

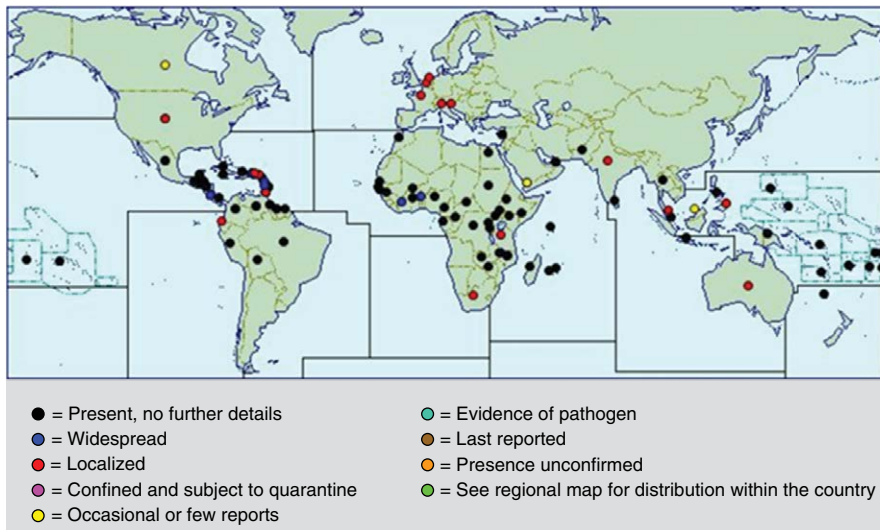


Fig. 17.6. Global distribution of *Radopholus similis*. (From CAB International.)

are too low for *R. similis*. Its worldwide distribution and strong association with export bananas has led to it being viewed as the most important nematode problem affecting banana. Its distribution has essentially resulted from the transfer of infected plant material, which appears to be correlated with the areas where banana plants of the subgroup Cavendish (AAA) were imported. The host range of *R. similis* has widened, further to its exposure to different plant species.

Symptoms of damage

The most obvious symptom of attack by *R. similis* on banana is the dramatic toppling of heavily infected plants, involving the uprooting of plants (see Fig. 17.5a and b), especially those bearing fruit, and especially following strong winds (though not necessarily). However, there is a range in gradation in the severity of damage, from the inconspicuous lengthening of the vegetative cycle to the drastic reduction in bunch weight due to reduced water and nutrient uptake. At a more macroscopic level, nematode infection results in dark red lesions on the outer part of the root, which penetrates throughout the cortex but not in the stele (Fig. 17.7). Adjacent lesions may coalesce and the cortical root tissue atrophies, ultimately turning black, killing the roots, which become withered. Secondary infection by

fungal pathogens is often associated with nematode infections. In heavy infections, the lesions girdle the roots. Infected roots may also have a cracked appearance. Nematodes can migrate from infected roots into the corm, causing diffuse black lesions, which may then spread around the corm (Fig. 17.8). Roots emerging from the corm become infected as they grow out. Toppling occurs commonly during windstorms or when heavy rains loosen the soil. The mechanical stresses on the root system are often increased by the natural angle of leaning, which develops as fruit bunches grow. Pseudostem girths and leaf cover can also be reduced (Roderick *et al.*, 2012a), while pseudostem turgidity can be affected, especially under periods of water stress, leading to them snapping more easily (Coyne *et al.*, 2013). The presence of a number of fungi in nematode-induced lesions, which will colonize the stele, undoubtedly hastens the destruction of roots, contributing to reduced anchorage and toppling (Sikora and Schlosser, 1973; Stover, 1972).

Biology and life cycle

R. similis is a migratory endoparasite that completes its life cycle within the root cortex. Penetration occurs mostly near the root tip, but nematodes can invade along the entire length of



Fig. 177. Lesion nematode infected banana roots sliced longitudinally (a) and whole, showing necrosis, which penetrates throughout the cortex but not in the stele (b). (Photographs courtesy of D. Coyne.)



Fig. 178. *Radopholus similis* infected banana corm showing areas of necrosis. (Photograph courtesy of D. Coyne.)

the root; females and all juvenile stages are infective; males are morphologically degenerate (with poorly developed stylets), and are probably non-parasitic. After entering the roots, the nematodes occupy an intercellular position in the cortical parenchyma, where they feed on the cytoplasm of nearby cells, causing cavities, which then coalesce to appear as tunnels. Invasion of the stele is not observed, except occasionally on very young roots (Valette *et al.*, 1997). Females move through the roots in search of healthy tissue. Females lay their eggs within infected tissues, with an average of 4–5 eggs/day for 2 weeks. The complete life cycle takes 20–25 days between 24 and 32°C; the eggs hatch after 8–10 days and the juvenile stages are completed in 10–13 days (Loos, 1962). It does not reproduce below 16–17°C or above 33°C (Fallas and Sarah, 1995; Pinochet *et al.*, 1995).

Pathotypes/races/biotypes

R. similis was considered to have two races, one attacking 'banana' but not citrus, and a 'citrus race' pathogenic to both. These two races were controversially separated into two species with the description of *Radopholus citrophilus* (Huettel *et al.*, 1984), which has since been viewed as two pathotypes of the same species (Valette *et al.*, 1998; Kaplan *et al.*, 2000; Haegeman *et al.*, 2010).

Physiological differences in reproductive capabilities and morphological variations of *R. similis* on banana indicate that different biotypes or isolates exist, based on host preferences and the rate of reproduction (Pinochet, 1979; Tarte *et al.*, 1981; Kaplan and O'Bannon, 1985; Hahn *et al.*, 1996; Marin *et al.*, 1999; Stoffelen *et al.*, 1999; Dochez *et al.*, 2005, 2009, 2013; Plowright *et al.*, 2013). In Uganda, *R. similis* populations collected from four locations demonstrated differences in their reproductive fitness, which was considered important in determining the potential pathogenicity of populations for use in resistance screening (Dochez *et al.*, 2013). Plowright *et al.* (2013) assessed the pathogenic and genetic variability of seven populations of *R. similis* originating from banana in Uganda and demonstrated that their virulence differed and displayed pathotype-like variation that could be grouped into two putative genomic groups, which corresponded with *R. similis* relative pathogenicity. Most of these populations from Uganda were able to reproduce and damage roots on the two widely confirmed sources of resistance, Yangambi km5 and Pisang Jari Buaya. Using RAPD techniques, they were able to detect

putative markers for nematode virulence. The distribution of these *R. similis* populations appears to be linked to the movement of planting material. In the case of Uganda, the aggressive populations appear, on the basis of phylogenetic analysis, to originate from Sri Lanka (Plowright *et al.*, 2013). These types of studies are important for future nematode management programmes based on resistance.

Survival and means of dissemination

R. similis survives in soil on infected banana roots and corms for substantial periods of time, as well as on some weed hosts. Unlike some other species, *R. similis* has no specialized survival strategy outside of the host. Tarjan (1961) and Loos (1961) demonstrated that *R. similis* did not survive in the soil for more than 6 months in the absence of host roots or pieces of live corms. Within planting material, it is the major means of re-infection and the principal mode of dissemination. The survival of *R. similis* in field soil during the intercropping cycle was studied in Martinique by Chabrier *et al.* (2010). In both Andosols and Nitisols, survival was significantly higher for males than for females. After 180 days, 21.7% of males and 9.8% of females remained alive, whereas no juveniles survived after 150 days. The relatively long survivorship of males enables them to fecundate females without competing for food after becoming adults.

The passive dispersal of the nematode in runoff water and through irrigation is potentially serious in areas where infested banana fields are adjacent to new plantings. In the studies of Chabrier *et al.* (2010), survival was much lower in water and soil solution than in natural soil. The mean half-life of males was 8.8 days, and 6.2 days for females. Just 8% of males and females from the initial population survived after 35 days.

Other hosts of Radopholus similis

Most edible *Musa* cultivars are attacked by *R. similis* (Luc and Vilardebó, 1961; Wehunt *et al.*, 1978; Davide and Marasigan, 1985), as well as abacá (Taylor and Loegering, 1953) and other seeded *Musa* species. In the Americas, *R. similis* seems to be confined to *Musa* spp. and to some cultivated plants, including ornamentals such

as *Anthurium andraeanum* (Bala and Hosein, 1996; Quénéhervé *et al.*, 1997; Sipes and Lichty, 2002). More than 250 plants have been reported susceptible to *R. similis*, including several crops of global economic importance, as well as weeds (O'Bannon, 1977; Bridge, 1987; Quénéhervé *et al.*, 2006b). Its status has been studied extensively from a quarantine point of view (Ayala and Roman, 1963; Edwards and Wehunt, 1971).

Pratylenchus

Several species of *Pratylenchus* root lesion nematodes are reported attacking *Musa* spp. throughout the world (Fig. 17.9). Of these, *Pratylenchus coffeae* and *P. goodeyi* are recognized as the most damaging. In South Africa, *P. brachyurus* is also regularly found in addition to the more common *P. coffeae* (Daneel *et al.*, 2015). However, many reports of *Pratylenchus* from banana refer to genus only, without identifying species, due on the one hand to limited taxonomic expertise but also to the difficulty in identifying *Pratylenchus* species. Indeed *P. coffeae sensu lato* is viewed as a 'complex' of morphologically similar but distinct species (e.g. Duncan *et al.*, 1999). This is highlighted by the case of *P. coffeae* affecting plantain in Ghana (Brentu *et al.*, 2004), which has since been redescribed as a separate but morphologically similar species, *Pratylenchus speijeri*, using molecular characterization (De Luca *et al.*, 2012).

P. coffeae is a pan-tropical species first observed in plantain roots in Grenada and described as *Tylenchus musicola* by Cobb in 1919. This species is very prevalent in the Caribbean, and *P. coffeae* is becoming increasingly dominant across West Africa, primarily on plantain, replacing *R. similis*, and increasingly prevalent across East and southern Africa on banana (Coyne, 2009; Dubois and Coyne, 2011).

P. goodeyi was first observed in banana roots in the Canary Islands by De Guiran and Vilardebó (1962), together with *P. coffeae* and *Pratylenchus thornei*. *P. goodeyi* is present, and the dominant nematode, in many banana-growing areas of East and Central Africa at elevations over 1400 m altitude, where temperatures are lower, as well as on Mt Cameroon above 700 m (Speijer and

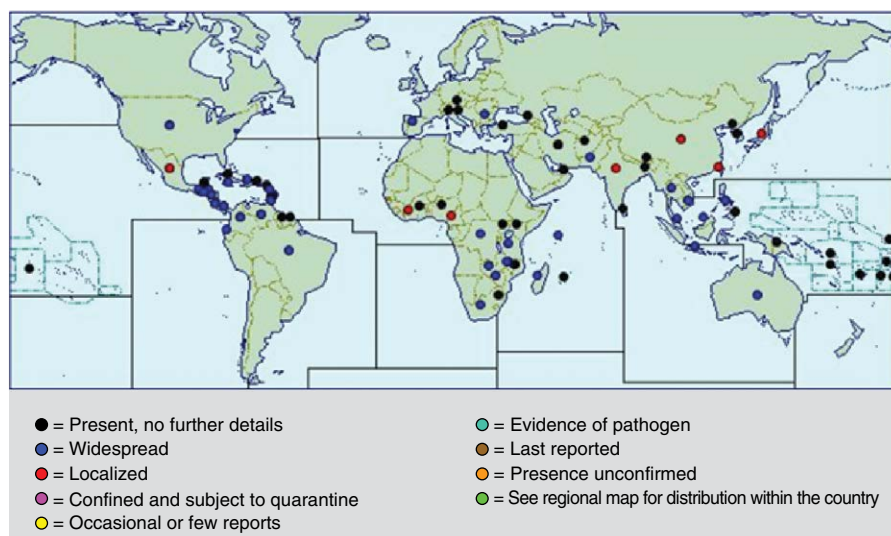


Fig. 179. Global distribution of *Pratylenchus coffeae*. (From CAB International.)

Fogain, 1999), indicating that it is indigenous to sub-Saharan Africa at high, cooler elevations (Price and Bridge, 1995). More recently, however, it has been detected increasingly from banana in lowland, tropical climates in East Africa from morphological identifications (Ses-hu Reddy *et al.*, 2007; Coyne, 2009; Luambano *et al.*, 2017). In Rwanda, *P. goodeyi* was the dominant species in East African highland banana fields across a wide range of agroecological conditions, altitudes and soil types (Gaidashova *et al.*, 2009). Although its presence was correlated significantly with root necrosis, no correlation could be established between incidence of *P. goodeyi* and cooking banana losses. In Tanzania on banana (Speijer and Bosch, 1996) and on enset in Ethiopia (Peregrine and Bridge, 1992), however, the extremely high densities of *P. goodeyi* found would indicate significant damage to production, even though losses were not recorded.

P. goodeyi is regarded as a major pest in commercial Cavendish plantations in the Canary Islands (De Guiran and Viledardebó, 1962), and has also been recorded in Madeira, Egypt and Crete (Gowen *et al.*, 2005). In Australia, *P. goodeyi* was as pathogenic as *R. similis* in commercial Cavendish plantations in subtropical areas, with *P. goodeyi* more prevalent in the cooler months and *R. similis* during the warmer

months (Pattison *et al.*, 2002). It is also common on banana in Hainan Province, China (Zhang *et al.*, 2015). Abacá is a host of *P. goodeyi* (O'Bannon, 1975). In Ethiopia, *P. goodeyi* is the predominant nematode species on enset, across enset-growing agroecological zones, affecting all 71 enset cultivars sampled, to varying degrees (Bogale *et al.*, 2004; Addis *et al.*, 2006).

Symptoms of damage

Root lesion nematodes cause symptoms of damage similar to those of *R. similis*: stunting of plants; lengthening of the vegetative cycle; reduction in size and number of leaves and in bunch weight; reduction of the productive life of the plantation; toppling and snapping when water stressed (Brentu *et al.*, 2004; Coyne *et al.*, 2013). Roots heavily infected by *P. coffeae* have extensive black or purple necrosis of epidermal and cortical tissue, often accompanied by secondary rotting and root breakage. Similar necrosis can be observed on the outer parts of the corm (Bridge and Page, 1984). In the Canary Islands, *P. goodeyi* penetrates the cortical parenchyma of banana roots, forming small, brownish-red, elongated flecks (De Guiran and Vilardebó, 1962). These feeding areas enlarge and eventually coalesce, so most of the cortical parenchyma is destroyed, impairing root function.

Biology and life cycle

P. coffeae and *P. goodeyi* are migratory endoparasites of the root cortex and banana corm. Nematodes of both sexes and all juvenile stages are invasive. The life cycle is completed within the root. Pinochet (1978) described the histological changes after inoculation of *P. coffeae* on roots of AAB clones. After entering the roots, the nematodes migrate between and within the cells, occupying a position parallel to the stele. They feed on the cytoplasm of neighbouring cells, eventually causing cavities that coalesce. The destruction of the cortical parenchyma of plantain roots by *P. coffeae* was very similar to those effects described by Blake (1963, 1966) for *R. similis* on dessert bananas, except there was no cell enlargement or increase in size of cell nucleus or nucleolus. The life cycle has been discussed in detail on other host plants (Zimmerman, 1898; Gotoh, 1964), and the average life cycle takes about 27 days at 25–30°C. *P. speijeri* is anticipated to have a similar life cycle as that of *P. coffeae*.

Pathotypes/races/biotypes

There is scarce information on 'biotypes', 'isolates' or 'races' of *P. coffeae*. Wehunt and Edwards (in Stover, 1972) mention different biotypes or isolates from Honduras and Panama, stated in terms of host preferences related to the infection index on test plants of abacá, plantain and banana. Morphological and genomic variation between 32 isolates of *P. coffeae* and closely related species has led to different groupings within the *coffeae* group (Duncan *et al.*, 1999). The relatively rapid increase in the incidence and distribution of *P. coffeae* in East Africa is intriguing, especially as it has traditionally not been a common nematode in the region. Similarly, the occurrence of *P. goodeyi* in lowland banana fields, outside of its normal temperature range, raises questions as to its accurate identification or the development of temperature-based biotypes. More molecularly based assessments are necessary.

Survival, other hosts and means of dissemination

Root lesion nematodes infect the corm, and consequently can be readily disseminated in the same way as that for *R. similis*. Records of

dissemination on infected corms are reported from Côte d'Ivoire for *P. coffeae* on dessert banana and plantain (Adiko, 1988; Fargette and Quénéhervé, 1988) and from East Africa for *P. goodeyi* on highland banana (Bridge, 2000). Numerous hosts of *Pratylenchus* spp. are documented, several of which may be weeds (Fluiter and Mulholland, 1941; Kaplan and MacGowan, 1982; Quénéhervé *et al.*, 1995). *P. coffeae* is also a major pest of various economically important crops, including tuber crops, ornamentals and tree crops (Pinochet and Duarte, 1986; Bala and Hosein, 1996; Quénéhervé *et al.*, 1997).

In a field study in Cameroon, Jacobsen *et al.* (2009) demonstrated that some bean and maize cultivars were good hosts of *P. goodeyi*; watermelon and onion were considered intermediate hosts; taro, okra, solanum potato and sweet potato were poor hosts, while cocoyam and tomato were very poor hosts. There was variation in host status depending on maize cultivar.

Helicotylenchus multicinctus

After *R. similis*, the spiral nematode *H. multicinctus* is probably the most widespread and abundant nematode on banana. *H. multicinctus* and *R. similis* are often encountered together in many dessert banana-growing regions of the world, particularly where banana is grown under optimal conditions. *H. multicinctus* is often regarded as the main parasitic nematode on banana where environmental conditions are sub-optimal for the crop (and also for *R. similis*) in relation to latitude, temperature and rainfall (McSorley and Parrado, 1986). In West Africa, *H. multicinctus* regularly occurs in combination with *Meloidogyne* spp. on plantain and dessert banana, where it can occur in very high densities and be highly damaging (Caveness and Badra, 1980; Adiko and N'Guessen, 2001; Sawadogo *et al.*, 2001). Spiral nematodes have also been found infecting abacá (Bridge, 1976) and enset (Addis *et al.*, 2006).

Symptoms of damage

The nematodes attack and feed on the outer cells of the root cortex and produce small, characteristic necrotic lesions (Luc and Vilardebó, 1961). The development of root lesions caused by

H. multicinctus is slow, relative to those produced by *R. similis*. Lesions on primary roots tend to remain shallow and superficial, appearing as numerous small dashes, reddish-brown to black in colour. However, in heavy infections, those lesions can coalesce, causing extensive root necrosis in the outer cortex, and dieback; lesions can also be found in the corm (Quénéhervé and Cadet, 1985). As *H. multicinctus* is found primarily occurring in combination with other parasitic species, it is difficult to 'separate' the damage it causes, with damage by more aggressive species eclipsing the damage by *H. multicinctus*. The effects of *H. multicinctus* on both banana and plantain can lead to stunting of plants, lengthening of the vegetative cycle, reduction in the size of the plant and in bunch weight, and reduction of the productive life of the plantation (Ssango *et al.*, 2004). Toppling may also occur in situations where there are heavy infestations, as well as snapping during water stress (Coyne *et al.*, 2013).

Biology, life cycle and survival

H. multicinctus, unlike other *Helicotylenchus* species, feeds endoparasitically on banana, completing its life cycle within the cortical part of the root, where both sexes and all juvenile stages, including eggs, can be found (Zuckerman and Strich-Harari, 1963). The host-parasite relationships of *H. multicinctus* were studied by Blake (1966), who observed that 4 days after inoculation of banana roots, the nematodes were wholly embedded within the cortex, sometimes to a depth of four to six cells. Nematodes fed on the cytoplasm of the surrounding cells in the root cortex. Infected tissues show various types of cellular damage, such as contracted cytoplasm, distorted or ruptured walls and enlarged nucleus but, in contrast to those observed with *R. similis*, histological changes are confined to parenchyma cells close to the epidermis. Damaged cells were often discoloured and became necrotic (Orion *et al.*, 1999). To date, there is no available information on 'biotypes', 'isolates' or 'races' of *H. multicinctus*. Little information is available on the survival of *H. multicinctus* in the absence of a susceptible host. As with *R. similis*, survival occurs on infected corms or on tissue remaining from the previous crop. Infected planting material is also the main means of dissemination.

Other hosts of Helicotylenchus multicinctus

Most edible *Musa* cultivars are attacked by *H. multicinctus* (Luc and Vilardebó, 1961; Gowen, 1976; Zem *et al.*, 1981; McSorley and Parrado, 1983). This nematode is also recorded to have a wide host range, including numerous crop species (Goodey *et al.*, 1965; Stoyanov, 1967), as well as weeds in banana fields (Quénéhervé *et al.*, 2006b).

Meloidogyne

Root knot nematodes are worldwide in distribution, attacking a vast array of economically important crops. On banana, its importance may have been underestimated because of the emphasis on the damage caused by lesion nematodes, by inappropriate sampling and extraction procedures (intended for lesion nematodes) and by the technical problems of apportioning crop loss in mixed infections. Damage has been noted particularly in greenhouse production systems in North Africa and the Canary Islands (Pinochet *et al.*, 1998). The species most commonly found associated with banana and plantain are *Meloidogyne incognita*, *Meloidogyne arenaria*, *Meloidogyne javanica* and *Meloidogyne hapla*. Different mixed *Meloidogyne* spp. can be observed in the same gall (Pinochet, 1977), as in West Africa (Netscher, 1978; Fargette, 1987), in the Caribbean and French Guiana (Cofcewicz *et al.*, 2005) and Brazil (Cofcewicz *et al.*, 2001). These are the most abundant root knot nematode species in banana roots in South Africa (Daneel *et al.*, 2015), in Taiwan (Lin and Tsay, 1985) and in north Yemen (Sikora, 1979) causing damage to banana plants. Root knot nematodes are being viewed as more important pathogens than previously, such as in Brazil, where they have occurred in 79% of root samples (Cofcewicz *et al.*, 2005) and in the Democratic Republic of the Congo, where they are present in 48% of fields in the lowlands and 61% of fields in the highlands (Kamira *et al.*, 2013). In South Africa and West Africa, they are common in combination with *H. multicinctus* (Caveness and Badra, 1980; Adiko and N'Guessan, 2001; Sawadogo *et al.*, 2001; Daneel *et al.*, 2015). In Malaysia, they were the most predominant species recovered (Rahman *et al.*, 2014), and in Vietnam, together with *P. coffeae*, they were the major

nematodes associated with banana (Van den Bergh *et al.*, 2006). Root knot nematodes are common throughout banana-growing regions of Australia, but have not been associated with yield loss (Stanton, 1994).

Root knot nematodes affect abacá in the Philippines and onset in Ethiopia (Addis *et al.*, 2006). *Meloidogyne graminicola* and *Meloidogyne enterolobii* cause galling on banana in Fujian Province, China (Zhou *et al.*, 2015, 2016).

Symptoms of damage

The most obvious symptoms of root knot infection are galling on primary and secondary roots (Fig. 17.10a), sometimes causing them to bifurcate and distort. Swellings, as opposed to galling, may often be observed, especially on cord roots. When sliced lengthways, females can be observed within the swellings, sometimes as darkened or necrotic areas, surrounding the females (Fig. 17.10b). Stunted growth has been attributed to root knot nematodes in India (Sudha and Prabhoo, 1983) and Taiwan (Lin and Tsay, 1985). Differences in symptoms can vary depending on *Meloidogyne* species and cultivar. Sikora (1979) observed higher levels of root rot in plantations in Yemen where *M. incognita* and *Fusarium solani* or *Rhizoctonia* sp. were concomitantly present.

Biology and life cycle

The life cycle, histopathology and aetiology of root knot nematodes do not differ significantly

on bananas from those reported on other hosts (see Perry *et al.*, 2009). In thick, fleshy primary roots, egg masses may not protrude outside the root surface, and multiple cycles can be completed within the same root. In mixed nematode infections, the area of influence of this nematode can start 60–90 cm from the rhizome, due to *R. similis* suppressing or replacing *Meloidogyne* (Luc and Vilardebó, 1961; Pinochet, 1977; Quénéhervé, 1990).

Survival, other hosts and means of dissemination

As with other banana nematodes, the survival and dissemination of root knot occurs with infected planting material (Quénéhervé and Cadet, 1985). Because of the wide host ranges of root knot nematodes, associations with weeds in banana plantations are more numerous than for other major nematode parasites. Special attention would be needed in maintenance fallows or in the selection of cover crops or associate crops in intercropping systems.

Rotylenchulus reniformis

Since the first records of *R. reniformis* on banana in Puerto Rico by Ayala and Roman (1963), this nematode is now recorded from numerous areas, including South America, Hawaii, the Caribbean, Africa, Asia and the Mediterranean (Coyne and Kidane, 2018). The life cycle and the histopathology

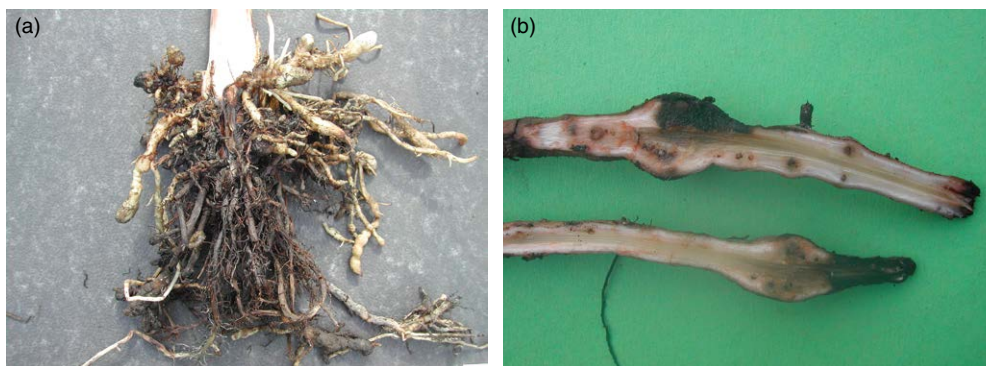


Fig. 17.10. *Meloidogyne incognita* roots showing galling on primary and secondary roots of banana (a); white females embedded in swollen roots, with necrotic tissue surrounding females (b). (Photographs courtesy of D. Coyne.)

and aetiology do not differ significantly on bananas from those reported on other hosts (Sivakumar and Seshadri, 1974). Juveniles of *R. reniformis* are commonly recovered from the soil, and permanent feeding positions occur mostly on the secondary roots (Ayala, 1962; Edmunds, 1968). As for *Meloidogyne* spp., the effect of this nematode is probably influenced by the presence of other root parasitic nematodes.

Other nematodes

Of the many other species of plant parasitic nematodes associated with bananas, some are considered damaging locally, but often without conclusive evidence to show their pest status. Invariably, these nematodes are in mixed communities with species already established as key pests.

Hoplolaimus pararobustus, a large endoparasitic feeding nematode, occurs around and within the roots and corms of dessert banana and plantain in West Africa (Quénéhervé and Cadet, 1985; Adiko, 1988; Quénéhervé, 1989a,b; Price, 1994). Root densities as high as 200 individuals/g root in mature plants have been observed (Mateille *et al.*, 1988b), which would most likely cause discernable damage by such a large nematode. Coyne *et al.* (2013), in field trials in Nigeria on plantain cv. Agbagba, demonstrated significant damage, leading to productivity loss, with bunch weights reduced by 53% in the first crop cycle.

Helicotylenchus mucronatus and *Helicotylenchus microcephalus* have been found causing root necrosis and stunted growth of banana at separate sites in Papua New Guinea (Bridge and Page, 1984). *Cephalenchus emarginatus* were found at densities up to 9000/l soil taken from around the roots of dessert banana and plantain in Côte d'Ivoire (Adiko, 1988; Mateille *et al.*, 1988b; Quénéhervé, 1989a,b). *Heterodera oryzicola*, a pest normally associated with rice, occurs on banana where these crops grow together. Its pathogenicity has been demonstrated (Charles and Venkitesan, 1993).

A. ensete affects enset in Ethiopia, causing characteristic black streaks on leaves. Severe infection can result in seedling and sucker death, and the premature death of leaves on more mature plants.

Environmental factors affecting parasitism of banana nematodes

Under humid, tropical conditions, the major factors affecting banana nematodes are abiotic, such as soil type and climate, and biotic, such as plant host status, growth stage, competition with other nematode species and other pests. In subtropical or highland countries, soil temperature is an additional factor influencing parasitism. Unthriftness of the crop may result from shallow or poorly drained soils, drought, nutrient deficiency or nutrient imbalance. Such conditions may restrict root development, and in these situations, the presence of nematodes may increase the incidence of toppling, as well as exacerbate foliar symptoms. If drainage is poor, high or fluctuating water tables can curtail root growth considerably (Lassoudière and Martin, 1974). Roots in soil saturated for more than 24 h die and rot rapidly. The combination of poor drainage and a nematode problem may result in nematodes and roots being concentrated in the upper layer of soil, resulting in more severe nematode damage.

Influence of soil type

The influence of soil type on nematode community composition has been reviewed by Ferris and Ferris (1974), and Vrain (1986) reviewed the effect of soil moisture content on population dynamics. Traditionally, most information concerning the relationship between soil type and nematodes focused on commercial bananas (e.g. Stover and Fielding, 1958; Ayala and Roman, 1963; Davide, 1980), although this has changed with more information becoming available for smallholder and cooking-banana systems (Gaidashova *et al.*, 2009). In Côte d'Ivoire, Quénéhervé (1988) showed that, in an organic soil, *H. multicinctus* was predominant in both soil and roots, while on mineral soils *R. similis* predominated. The major differences in nematode community structure occur in the soil. *R. similis* seems less affected by soil variables because it is strictly an endoparasite. *H. multicinctus* is more frequent in soils characterized by high levels of clay, silt or organic matter and low pH. *H. pararobustus* is more commonly found in coarse volcanic or sandy soils, and *Meloidogyne* spp. is most abundant in sandy soils.

Influence of climatic factors

Numerous studies have attempted to relate population densities with climatic factors, particularly rainfall; in general, it is assumed that conditions promoting plant root growth also favour nematodes. Most extended studies of population dynamics have shown a decline in *R. similis* densities during the wet season (Jimenez, 1972; Melin and Vilardebó, 1973; Jaramillo and Figueroa, 1974; McSorley and Parrado, 1981; Hugon *et al.*, 1984; Hunt, in Ambrose, 1984; Quénéhervé, 1989a,b), but the opposite effects have also been reported (Marcelino *et al.*, 1978; Davide and Marasigan, 1985). Similar attempts have been made to correlate *H. multicinctus* densities with rainfall, with inconsistent results (Hutton, 1978; McSorley and Parrado, 1981; Badra and Caviness, 1983; Quénéhervé, 1989a,b) but densities generally increase during the wet season. The discrepancies in the relationships between population densities and rainfall may be attributed to the difference in soil type, soil temperature, incidence and intensity of rainfall and root growth. Consideration should be given to sampling procedures before initiating such nematode population dynamic studies.

Influence of the root system and physiology of the plant

Successive annual peaks in *R. similis* root densities have been related to the active growth of the plant (Jaramillo and Figueroa, 1974), which coincides with the emergence of the banana flower (Melin and Vilardebó, 1973). In Guadeloupe, Hugon *et al.* (1984) observed a relationship between the physiological stage of the banana plant and climatic factors (e.g. temperature and rainfall). Pruning of excess suckers is practised in commercial plantations, and this may influence the relative densities of *R. similis* and *H. multicinctus* in the roots and corms (Mateille *et al.*, 1984). Quénéhervé (1989a,b) shows differences in the behaviour of the nematodes encountered and that *R. similis* acts as the primary root invader, with levels of infection decreasing as the root system ages or decays. Blake (1961) and Loos (1962) show that migration and egg laying are governed by nutritional factors and that the 'nematodes do not move out of a root so long as they are able to invade healthy tissue'. *R. similis* is

able to complete its life cycle in the cortical tissue of the root or the rhizome without a soil phase. After flowering, there is no new root emergence from the main rhizome (Lavigne, 1987), but on the rhizomes of leading suckers, prolific root emergence occurs. In fact, all the factors, endogenous or exogenous, that favour root emergence on banana plants contribute to the build-up of *R. similis* (Quénéhervé, 1993a).

Influence of the competition with other parasites

Infections by nematodes will accelerate root decay with one species restricting the availability of healthy tissue to another, but which may depend on the conditions. *H. multicinctus* and *R. similis* often occur together on banana and plantain, although Vilardebó and Guérout (1976) have noticed that high densities of *H. multicinctus* build up when *R. similis* is locally absent. In Côte d'Ivoire, it appears that on organic soil, *H. multicinctus* densities may surpass those of the primary invader *R. similis*. *P. coffeae* has a similar parasitic behaviour to *R. similis* and may compete directly with it, as appears to be the case in West Africa, where it has mostly replaced *R. similis* (Coyne, 2009). In some parts of the world, this nematode might be the more damaging parasite, such as in Papua New Guinea, or in West Africa, or like *P. goodeyi* in the Canary Islands (De Guiran and Vilardebó, 1962) or on highland bananas in East Africa (Bridge, 1988; Kshaija *et al.*, 1994). The banana weevil, *Cosmopolites sordidus*, can confuse the diagnosis of a nematode problem because symptoms of damage are similar. Pathogenic fungi and bacteria also contribute to root decay. With fungi (*Cylindrocarpon* spp., *Fusarium* spp., *Rhizoctonia* spp. and *Cylindrocladium* sp.), the problem becomes complex, as nematodes and fungi occur within the same cells and infections result in the same types of discoloration and necrosis (Risède and Simoneau, 2004; Jones, 2018). Often, the problem is in defining the primary or major pathogen. Nematodes create a food base for weak, unspecialized fungal parasites, enabling them to invade the stele and to increase root necrosis. Differentiation is possible between the deep lesions due to *R. similis*, which are mainly associated with *Fusarium* sp., and the shallow and outer lesions of *H. multicinctus*, which are mainly

associated with *Rhizoctonia* sp. (Blake, 1963; Laville, 1964; Stover, 1966; Sikora and Schlosser, 1973; Booth and Stover, 1974; Pinochet and Stover, 1980). Those fungi acting as secondary parasites can increase root breakage, and consequently toppling. One of the most devastating fungal diseases affecting commercial banana, Fusarium wilt or Panama disease, caused by *Fusarium oxysporum* f.sp. *cubense*, increases in the presence of *R. similis* (Newhall, 1958; Loos, 1959), although this was not confirmed from work in the Philippines (Epp, 1987). Of the four races of *Fusarium* that affect edible banana cultivars, Tropical Race 4, presently found in India, China, Taiwan, the Philippines and Mozambique, is extremely aggressive and of extreme importance (Jones, 2018).

There is some evidence that root damage by nematodes facilitates increased incidence of Xanthomonas bacterial wilt (BXW) caused by *Xanthomonas campestris* pv. *musacearum* (Shehabu *et al.*, 2010). When East African highland cooking banana Pisang Awak and Matooke plants were inoculated with a combination of *P. goodeyi*, *Meloidogyne* spp., *Rotylenchus* spp. and *R. similis* and challenged 3 months later with *X. campestris* pv. *musacearum*, a significant increase in BXW incidence was observed when nematodes were pre-inoculated.

Economic importance

R. similis is widely regarded as the most important nematode species affecting *Musa*, and among the most important pathogens (Gowen *et al.*, 2005). Combinations of nematode species infect all smallholder fields and are an insidious production constraint in commercial plantations. In south-west Nigeria, however, *P. coffeae* was identified as the single most important bio-constraint to plantain production (Speijer *et al.*, 2001), while the same species has become increasingly dominant on banana and plantain across West Africa. Banana are rarely parasitized solely by a single nematode species though, and are regularly subjected to concomitant infection with other diseases or pests. The relative importance of each nematode species, therefore, is often difficult to determine and not always fully understood. Most evidence of crop loss from field experimentation comes from the use

of nematicides, which usually reduce nematode densities and result in other beneficial plant growth effects. Yield responses reported with nematicide applications to dessert and cooking banana have reached 275% over untreated controls (see Tables 2 and 3 of Gowen and Quénéhervé, 1990). The differences in response undoubtedly result from several factors, in particular soil type, nematode species and biotype, and climate. Damage to plantain has reached 50% in experimental plots (Sarah, 1989; Fogain, 2001), including trials without using nematicides (Coyne *et al.*, 2013), demonstrating the severity of the problem. Although difficult to determine specifically, there is strong evidence that *R. similis* has contributed significantly to the decline of the East African highland banana in East and Central Africa since its introduction in the 1960s (Speijer and Kajumba, 1996; Speijer *et al.*, 1999; Price, 2006).

Management measures

Cultural practices

The opportunity for controlling *R. similis* with cultural techniques is somewhat limited in those areas where banana is grown continuously, without replanting. In replanted systems, control of *R. similis* can be undertaken through total destruction of the previous crop to eliminate the nematode, followed by a controlled fallow or rotation with non-host crops. As an introduced pest, the burrowing nematode *R. similis* may be absent from many areas not previously cultivated with bananas. Accidental introduction of the nematode is to be avoided, therefore, ideally using disease-free tissue culture plantlets. Macropropagated plantlets, when produced properly under sterilized conditions, are also suitable for smallholder systems in particular. However, smallholder farmers especially will continue to exchange and use suckers as planting material, which need to be disinfected.

Fallows may need to last 6 months or longer (Loos, 1961; Tarjan, 1961), and it is essential that all banana roots and suckers are destroyed, which in practice is very difficult. Fallowing is now widely practised where there is available land and *R. similis* is present. In the French Antilles, nematode control in commercial banana

plantations is based on sanitizing contaminated banana fields through chemical destruction of existing plants, a 1-year cultivated fallow, and replanting with tissue culture plants (Chabrier and Quénéhervé, 2003), which extends field longevity from 3–4 to more than 10 years, and almost eliminates *R. similis* (from 100% to less than 6.5% of infested banana fields with *R. similis* in Martinique). In Cameroon, when tissue culture plants were used after a 1-year fallow, very low levels of *R. similis* were recorded during the two cycles (15–18 months after planting) (Kashaija *et al.*, 1998). In Taiwan and India, rice may be grown in rotation with banana, and various rotation combinations have been evaluated in the French Antilles (Ternisien, 1989; Ternisien and Ganry, 1990). In South Africa, break cropping with non-hosts such as radish (*Raphanus sativus*), purple bean (*Phaseolus atropurpureus*), buffalo grass (*Megathyrsus maximus* var. *trichoglum*) and marigold (*Tagetes patula*) reduced *R. similis*, and in some cases also *Meloidogyne* spp., while sugarcane (*Saccharum* hybrid) eliminated *R. similis* after 10 weeks (Milne and Keetch, 1976; De Villiers *et al.*, 2007). Where banana is grown continuously, i.e. Latin America, or where it would be uneconomic to leave land fallow, crop rotation is uncommon. Interestingly, when including intercrops as an *R. similis* management strategy, preference should be given to intermediate and good *R. similis* hosts (e.g. common bean and sorgho-Sudangrass), since they result in lower nematode build-up and reduced (long-term) pathogen pressure in banana (van der Veken, 2010). Including a poor or non-host (e.g. marigold and sunn hemp) had an adverse effect on long-term nematode pressure, since they moved towards the banana, aggravating the problem.

In the French Antilles, management approaches that combined different tools for pest management, such as the use of improved fallows, crop rotations, the use of banana *in vitro* plants and tolerant cultivars, were effective in reducing nematode infestations and nematicide use. As an example, from 1996 to 2016, nematicide use on bananas in Martinique has decreased 97.3% in applied tonnage of active ingredients, and the banana treated surface per year has decreased from 100 to only 5.2%. However, the adoption of these innovative practices depends strongly on the farming contexts, and only

strong governmental incentive measures have succeeded in forcing banana growers to adopt these methods (Quénéhervé, 2017).

The system is being modified to use *R. similis*-resistant or less susceptible Cavendish clones and hybrids developed by the CIRAD breeding programme. This follows studies by Quénéhervé *et al.* (2011), who determined that the use of genetic and functional diversities of cultivars reduced nematodes and altered community composition. In the East African highlands, varietal mixtures is a common cropping practice and may be responsible for shifts in nematode species composition.

Beneficial results were obtained by flooding in Surinam and Côte d'Ivoire (Maas, 1969; Sarah *et al.*, 1983; Mateille *et al.*, 1988a) but this is now an uncommon practice.

Weeds can also be good hosts of many banana nematodes and need to be controlled when fallow is used as a control option; *R. similis* was consistently associated with plants in the Euphorbiaceae, Poaceae and Solanaceae in banana fields in Martinique (Quénéhervé *et al.*, 2006b).

The cultivation of banana with high-density planting systems is practised in a number of countries, especially by commercial growers, because of higher productivity and lower cost of production. When possible, non-infested fields are used, otherwise nematicides are used prior to planting. *R. similis* and *H. multicinctus* damage was reduced in ratoon banana grown under high-density planting systems using a combination of nematicides, neem cake and marigold incorporation (Seenivasan, 2017).

Organic amendments/mulching/ intercropping

Although there are conflicting opinions on the effects of mulching and organic amendments on the burrowing and lesion nematodes, mulched nematode-infested plots are likely to produce more than non-infested, non-mulched plots, because of the benefit of organic matter on plant growth. Intercropping with cover crops (Fig. 17.11) is sometimes considered to have repellent action against pests, and suppression of weeds, but failed to show any benefit in a 2-year study using leguminous crops in Uganda (McIntyre *et al.*, 2001).

In smallholder plantain fields heavily affected by nematodes in Nigeria, Tenkouano *et al.*



Fig. 17.11. Live mulching by intercropping *Geophila repens* as a ground cover in Porto Rico. (Photograph courtesy of P. Quénéhervé.)

(2006) demonstrated the beneficial effect of mulching suckers with organic matter (*Tithonia diversifolia*) to reduce losses due to nematodes and improve crop performance. In field trials, mulching with various organic materials, including wood chippings and oil palm refuse, reduced losses to nematodes and improved plantain yield performance (Rotimi *et al.*, 1999; Coyne *et al.*, 2005); in Rwanda, mulch significantly reduced root necrosis and *P. goodeyi* root densities on East African highland banana, leading to increased bunch weight in all mulched plots irrespective of root necrosis intensity (Gaidashova *et al.*, 2010). Basically, any mulch is better than no mulch (Fig. 17.12). In three soil types amended with a range of mulches, Pattison *et al.* (2011) found that amendments high in C and N appeared to induce suppression of plant parasitic nematodes by improving the environment for antagonistic organisms. When including a *Purpureocillium lilacinum* (syn. *Paecilomyces lilacinus*) based product, Bioact®, in addition to organic amendments, no effect of the treatments

on nematode density was observed in an established and a renewed plantation in Costa Rica (Chérrez and Pocasangre, 2010). Niere *et al.* (2014), also demonstrated that the application of mulch would not offset the detrimental effects of using nematode-infected sucker planting material of East African highland banana, but that healthy planting material provided significant benefits.

Physical treatments – hot water

Smallholder farmers especially will continue to exchange and use suckers for planting, for which adoptable disinfection methods are necessary. The simplest recommended practice is to remove the external tissue of the corm, together with root stumps and adhering soil, by peeling (paring) until only the white corm tissue is exposed (Fig. 17.13). The paring of suckers should be conducted away from the field, and severely lesioned corms discarded. Suckers should then be heat treated, as paring will never totally remove



Fig. 17.12. Effects of mulching on nematode-infected plantain (rear plot) in the second ratoon in Nigeria, compared with no mulch. (Photograph courtesy of D. Coyne.)

all nematode infection. Immersing banana suckers in hot water (55°C for 15–25 min) is effective for large-scale commercial settings and is practised in Australia and Central and South America (Stover, 1972), but is impractical for smallholders. The adapted boiling water technique (Hauser and Coyne, 2010), however, has proved effective and suitable for smallholder adoption (Hauser and Messiga, 2010). Boiling water treatment for 30 s was optimum for East African highland banana and plantain, and reduced combined nematode densities of *H. multicinctus*, *R. similis* and *Meloidogyne* spp. to a fraction of farmer controls (Coyne *et al.*, 2010; Hauser and Messiga, 2010).

Resistance and tolerance

No widely grown clone of export banana is known to be resistant to the important nematodes, while genetic improvement in the past has been hindered by the complexity in breeding new banana cultivars (Menendez and Shepherd, 1975; Ortiz *et al.*, 1995). New cultivars need to have the necessary agronomic and fruit quality attributes to meet the demands of the export

trade. Techniques for exploiting genetic resources have been developed (Persley and de Langhe, 1987; Ganry, 1993; Atkinson *et al.*, 2004; Roderick *et al.*, 2012b; Nyine *et al.*, 2017). The difficulty for nematologists, however, is that no gene(s) for resistance have yet been recognized that can provide a basis for a systematic breeding programme. Nor have nematodes been the first priority for breeders, who traditionally have focused on developing hybrids with resistance to Black Sigatoka (*Mycosphaerella fijiensis*) and Fusarium wilt (Ganry, 1993). The crisis caused by Panama disease, which led to the change of export cultivar from Gros Michel to Cavendish, exacerbated the problem of *R. similis*. Dedicated breeding for resistance to *R. similis* began in Honduras after the discovery of resistance in the diploid cv. Pisang jari buaya (Wehunt *et al.*, 1978), which was subsequently used in hybridization programmes that resulted in an improved hybrid breeding clone SH3142 (*Musa* AA) with resistance to *R. similis* (but not *Pratylenchus*) (Pinochet and Rowe, 1979; Pinochet, 1988). SH3142 has many qualities required by banana breeders and was largely used by the Fundación Hondureña de Investigación Agrícola



Fig. 17.13. Preparing suckers for planting: paring plantain suckers in Nigeria (a) and dessert banana in Porto Rico (b). (Photographs courtesy of D. Coyne and P. Quénéhervé.)

(FHIA) programme (Rowe and Rosales, 1994; Ortiz *et al.*, 1995). The tetraploid FHIA 1 (*Musa* AAAB), which has SH3142 parentage, consistently failed to demonstrate the same level of resistance in the field, however (Stanton, 1999).

Over the past decade or so, nematodes have increasingly become a focus for breeders, such as through the 'Breeding Better Bananas' initiative in East Africa between the national research programmes in Uganda, Tanzania and IITA (<http://breedingbetterbananas.org/>; accessed

19 December 2017) among others. Furthermore, it is important to note that the traditional focus on *R. similis* is steadily shifting towards identifying resistance against other nematodes. This is especially important in respect of *Pratylenchus* species, which are rapidly displacing *R. similis* and increasing in abundance, at least in Africa (Dubois and Coyne, 2011).

A whole range of resistance screening studies, using various procedures in the field and greenhouse, have been conducted and developed over the years (see Gowen *et al.*, 2005, Coyne and Kidane, 2018), often with contradictory results on the resistance status to nematodes of some important accessions. It can be difficult to compare results from these different screening procedures, due to highly variable environmental conditions and biological materials (plants and nematodes). There is evidence indicating that the screening of young *in vitro* plants may not be always reflective of results on older plants (Stanton, 1999). Although there are compelling reasons for conducting rapid screening trials on juvenile plants under glasshouse conditions (Speijer and De Waele, 1997; Quénéhervé *et al.*, 2006a, 2009a), it is clear that the final resistance assessments need confirmation from mature plants under field conditions. Since the numbers of hybrids produced through conventional banana breeding have been relatively small, arguably this is not such an important issue. However, breeding mechanisms are fast evolving, and improving throughput (e.g. Roderick *et al.*, 2012b; Nyine *et al.*, 2017; <http://breedingbetterbananas.org/>), which require more efficient and rapid screening methods to accommodate this improvement. De Schutter *et al.* (2001) developed the improved and more rapid single-root screening protocol, which was further developed for multiple species by Coyne and Tenkouano (2005), increasing screening efficiency. Despite these improvements, the method remains sensitive and prone to error, requiring further adjustments to create a more robust, rapid and efficient screening method.

In order to measure host reaction to *R. similis*, a number of parameters are assessed, measured and damage quantified, using various techniques devised over time. Trial designs, sampling strategies and methods of statistical analysis have been reviewed by Price and McClaren (1996), while Speijer and De Waele (1997) have

provided standardized protocols for nematode assessment damage on banana. To optimize and simplify the assessment of nematode host resistance on banana under field conditions, Hartman *et al.* (2010) identified an index using three key parameters: percentage of dead roots, number of large lesions on corms, and nematode root density.

Dochez *et al.* (2005) evaluated the host response to *R. similis* of the most commonly grown East African highland bananas in Uganda, as well as diploid hybrids and East African highland banana-derived hybrids. Except for cv. Muvubo, East African highland bananas were as susceptible to *R. similis* as the susceptible check cv. Valery; 4 of 13 tetraploid hybrids were identified with resistance to *R. similis*, as well as 13 diploids and 5 secondary triploids. Quénéhervé *et al.* (2009b) demonstrated that three banana hybrids (FB918, FB919 and FB924) (*Musa* spp., AAA group) had similar levels of resistance as the resistant control cv. Yangambi km5 against *R. similis*. Four hybrids (FB918, FB919, FB920 and FB924) also showed low multiplication of *P. coffeae* as in the resistant control. This study showed partial resistance to both *R. similis* and *P. coffeae* within synthetic hybrids of *M. acuminata*, adding an important extra value to these dessert banana hybrids formerly bred for resistance against *Mycosphaerella* leaf spot diseases. Tixier *et al.* (2008) additionally showed that where both *R. similis* and *H. multicinctus* were present, *H. multicinctus* would become dominant, with overall reduced damage to plants. The reactions of 19 new synthetic banana hybrids (primary tetraploids and improved diploids) to inoculation with *M. incognita* or *H. multicinctus* was studied under field conditions as well as in inoculation tests in pots (Das *et al.*, 2014a,b). Hybrid H 531 (Poovan, Pisang Lilin) exhibited resistance to both nematodes, and six hybrids were tolerant to both; H-03-17 and H 510 were also tolerant to *H. multicinctus*. The resistant and tolerant hybrids had enhanced contents of total phenol, PO, PPO and PAL. Das *et al.* (2014c) showed that the hybrid H 531 also had good combined resistance against fungal wilt and nematodes.

While resistance is one breeding objective, an additional consideration is crop structure and architecture, such as shorter plant height and larger, deeper penetrating root structures. One of several tetraploid AAAA genotypes developed by

the Banana Breeding Scheme in Jamaica derived from cv. Highgate, a mutant of Gros Michel, was found to be marginally less susceptible (Gowen, 1976), and casual observations indicated that tetraploids were less vulnerable to falling over in winds or wet weather. The relatively greater width of stems of some tetraploids, and perhaps more vigorous root systems, confer some tolerance to uprooting. The tetraploids produced by FHIA (Rowe and Rosales, 1994) also show relatively greater vigour than many triploid cultivars, but have the advantage of not being as tall as those produced in Jamaica.

Biochemicals that could be associated with resistance to *R. similis* and/or *P. goodeyi* include flavones, catechol, caffeic esters, ferulic acid, lignin, dopamine (Sarah *et al.*, 1997), flavonoids (Valette *et al.*, 1997, 1998), peroxidase (Mateille, 1994), phenalenone (Binks *et al.*, 1997), phytoanticipin-like compounds (Luis, 1998), condensed tannins, procyanidin and propylgallate (Collingborn *et al.*, 2000), total phenol (Das *et al.*, 2014a,b), pre-formed phenolic cells and lignified cell walls (Fogain and Gowen, 1996, 1998). Hölscher *et al.* (2014), using two cultivars differing in susceptibility to *R. similis*, demonstrated the nematicidal activity of the phenylphenalenone anigorufone, its ingestion by the nematode, and its subsequent localization in lipid droplets within the nematode.

In summary, there is resistance against *R. similis* in the Pisang jari buaya group, some other diploid accessions, such as Kunnan and some of the *M. acuminata* subspecies *burmanicoides* in India (Sathiamoorthy and Balamohan, 1993), and in the triploid *Musa* AAA Yangambi km5, which also has resistance to *P. goodeyi* (Fogain and Gowen, 1998). Resistance is apparent against *P. coffeae* in some cultivars belonging to the *burmanicoides* group and hybrids (Quénéhervé *et al.*, 2009a,b). There is some, but limited, evidence of resistance against *Meloidogyne* spp. and *H. multicinctus* (Das *et al.*, 2014a,b), and even combined resistance to both *R. similis* and *M. incognita* in Pisang Mas (Suganthagunthalam *et al.*, 2010). In commercial systems, a key objective is to reduce pesticide reliance and the obvious implication this has on environmental health, while for smallholder systems the focus is on reducing losses and improving farmer livelihoods. The nematode resistance in these cultivars will, however, be quite difficult to manipulate in

breeding improvement programmes, while the potential for transgenic nematode-resistant bananas edges ever closer (Shotkoski *et al.*, 2010; Tripathi *et al.*, 2015).

Chemical

Nematicides are widely used by commercial growers. They are, in many cases, the only tool available for consistent management of nematodes. However, dependence on nematicides has become less acceptable, towards a more integrated (and sustainable) system of pest management (Holderness *et al.*, 2000; Ganry, 2001). Consumer demand has also driven an organic banana trade. For smallholder producers, nematicide use is, for the most part, non-existent, due to the high cost of treatment and lack of knowledge on their benefits.

A unique number of factors make nematode management with nematicides challenging, such as the extremely large root system, the large volume of soil to be treated versus the limited soil surface to treat, the monoculture and perennial nature of the crop, the short duration of nematicide activity, accelerated nematicide microbial degradation and the ability of nematodes to recover or migrate from untreated areas to new emerging roots.

The method and timing of treatments varies according to cultural practices (Gowen, 1979), climate (Jaramillo and Figueroa, 1976), crop damage, and knowledge of nematode population dynamics and plant phenology (Quénéhervé *et al.*, 1991; Quénéhervé, 1993b). When planting new plantations using tissue culture plants or field-collected suckers (potentially nematode infected), the best results are achieved by applying nematicides to the planting hole (Gowen, 1979). Paring and nematicide dip treatment has been recommended (Broadley, 1979) but has not been used extensively. However, the replanting of banana fields is often uncommon, and nematicide treatments may begin on established infected crops. Consequently, the benefits of nematicide use may take several crop cycles to become apparent (Gowen, 1979).

Dosages for post-plant treatment in established plantations are usually applied on a per plant basis in a 35- to 100-cm radius around the plant, but not incorporated in the soil, to avoid

root damage. Established fields are generally treated with nematicides three times, or four times in heavily infested plantations, per year. In mature fields, the granular formulations may be sprinkled in a half circle around the selected follower sucker and not entirely surrounding the mother plant (Fig. 17.14).

Liquid concentrate formulations are used, including in drip irrigation systems, but this method of application does not have universal approval, for reasons of safety. In the Caribbean, oxamyl was used with a spot-gun spray applicator directly from disposable containers. A water-based formulation of a 10% concentration provides similar efficacy (C. Chabrier, Martinique, 2004, personal communication). Injection of oxamyl and furadan into the main pseudostem after harvest has led to increased bunch weight but no differences in nematode densities, when compared to soil treatment (Araya, 1999). Conversely, injection of nematicides into the follower sucker pseudostem avoided the biotic and abiotic inhibitory effects of the soil environment on the efficacy of nematicides reducing nematodes and increasing bunch weight (Araya, 2004a). Vargas *et al.* (2015), comparing plants injected with oxamyl and those receiving a rotation of nematicides on the soil surface, showed a higher percentage of functional roots, reduction in nematodes and improved plant growth compared to plants without treatment, but no differences between the nematicide treatments.

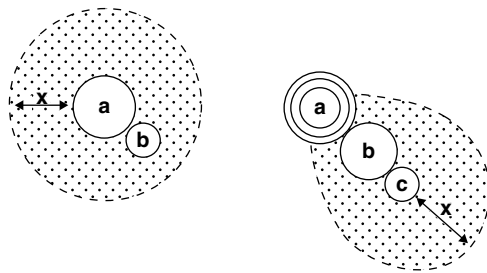


Fig. 17.14. Area of treatment when using granular nematicides on young banana plantations and on ratooning crops. (a) Mother plant; (b) selected daughter sucker (first ratoon); (c) selected daughter sucker (second ratoon); (x) radius of treatment area 35–50 cm.

Over time, the repeated use of certain nematicides can lead to enhanced and rapid microbial degradation (Smelt *et al.*, 1987; Suett and Walker, 1988; Anderson and Lafuerza, 1992). Cabrera *et al.* (2010a) demonstrated that terbufos efficacy lasted longer in soils with no or low history of terbufos application than in soils treated regularly, due to microbial breakdown by an array of Gram-positive and -negative bacteria *Brevundimonas*, *Burkholderia* and *Cupriavidus* (Cabrera *et al.*, 2010b). To avoid microbial degradation, rotating nematicides with different active ingredients is recommended (Araya, 2004b; Moens *et al.*, 2004).

The number of nematicides on the market fluctuates with time and countries, while application methods or improved combinations are continuously assessed. Vargas *et al.* (2006) reported that as the number of nematicide applications per year increased from 0 to 4, the mean density of nematodes, from the second to fourth cycles, also decreased significantly. On the contrary, Quénéhervé (2017) reports a decrease of nematicide applications per year from 2–3 to 0.3 in Martinique. Calvo and Araya (2005) established that doses of carbofuran, ethoprophos, terbufos and oxamyl higher than those recommended against *R. similis* had no additional effect, indicating that doses higher than those recommended was not justified. Salguero *et al.* (2017) demonstrated that three nematicide applications per year reduced total nematodes significantly, but that no difference was detected between two applications at 6- or 7-month intervals.

The fact that nematicides do not influence total nematode densities in the root over time is logical when the factors above are considered. The management effects of nematicide use are seen mainly in plant growth reaction and not in a reduction in nematode density. Many nematicides act in a nematostatic manner and therefore do not affect population densities significantly, and consequently need repeated applications. The development of precision application technology in which plants are treated individually at well-defined events could also contribute to a more integrated pest management system (Quénéhervé *et al.*, 1991).

In conclusion, the range of chemistry available for nematode control has declined over recent years as they have gradually been removed

from the market for safety and health reasons, without replacement. The situation is changing, however, and new products are emerging, but which may be locally specific and change over time. Current availability and recommendations therefore need to be consulted (see Chapter 23, this volume).

Biological control

Progress in discovering, characterizing and deploying antagonists of migratory endoparasitic nematodes, although not as great as for sedentary endoparasites, has advanced greatly and offers serious potential for nematode management in banana (Sikora *et al.*, 2008; Dubois and Coyne, 2011; Viaene *et al.*, 2013). This is especially true with regard to microbial enhancement of tissue culture plants (Sikora *et al.*, 2003; zum Felde *et al.*, 2005, 2009; Dubois *et al.*, 2011; Waweru *et al.*, 2013). Potential biocontrol agents include bacteria (Aalten *et al.*, 1998; Chaves *et al.*, 2009), mycorrhizae (Elsen *et al.*, 2003, 2008; Jefwa *et al.*, 2010; Olaniyi, 2014), *P. lilacinum* (Daneel *et al.*, 1998; Mendoza and Kiewnick, 2007) and mutualistic fungal endophytes (Sikora and Schuster, 1998; Sikora *et al.*, 2003, 2008; van Dessel *et al.*, 2011), including *Trichoderma atroviride* and non-pathogenic strains of *F. oxysporum* (zum Felde *et al.*, 2004; Niere *et al.*, 2004; Dubois *et al.*, 2006).

Endophytic colonization of banana plants affects nematode activity through a range of mechanisms, including the production of toxic metabolites and induced resistance pathways (Vu *et al.*, 2006; Paparu *et al.*, 2009, 2013; Schouten *et al.*, 2009). Sometimes, improved plant growth and vigour is observed following endophyte infection, even in the absence of nematode challenge (Fig. 17.15.). Host reactions, however, appear to be quite specific and can depend on a number of factors, including host cultivar, endophyte species or strain, including environmental conditions (Sikora *et al.*, 2007). This form of microbe–plant interaction offers huge potential towards improving our ability to manage nematodes, especially when combined with tissue culture planting material and possibly using long-term *in-planta* colonization of the mat for extended nematode antagonism



Fig. 17.15. Commercial tissue culture plantlets with and without plant growth and health-promoting fungal endophytes being hardened in the nursery for field transplantation in Costa Rica. (Photograph courtesy of L. Pocasangre and A. zum Felde.)

(Dubois *et al.*, 2006; Chaves *et al.*, 2009; zum Felde *et al.*, 2009).

Although much progress has been made, fostering an increasing interest, in the use of microbial antagonists, there is relatively limited data from field studies, especially regarding long-term activity. *P. lilacinum* has probably received most attention, and a number of commercially registered products based on this organism are now available for use on bananas, as well as other crops. In Kenya, Waweru *et al.* (2013, 2014) demonstrated screenhouse and field performance of three strains of the mutualistic fungal endophyte *E. oxysporum* against *P. goodeyi* and *H. multincinctus* on banana cvs. Giant Cavendish and Grand Nain. Growth increases up to 36% and yield increases of 20% were observed, providing resounding evidence on the benefits that endophyte enhancement of tissue culture plants can provide. The long-term effects of microbial enhancement, however, requires further attention. Sikora *et al.* (2010) demonstrated endophyte colonization of suckers developing from inoculated mother plants and the carry-over of *R. similis* suppression into

the next generation. Longer-term suppression over crop cycles is therefore possible when tissue culture plants are enhanced biologically with a well-matched endophyte or combination of endophytes.

The combination of biocontrol agents can improve host protection and growth, although this is not always the case, and can indeed be detrimental (Sikora *et al.*, 2010). Mendoza and Sikora (2009) tested multiple applications of antagonists having different modes of action against *R. similis*. Combinations of the mutualistic endophyte *E. oxysporum*, *P. lilacinum* and the antagonistic bacteria *Bacillus firmus* reduced *R. similis* root densities substantially, but only additively, not synergistically. The study demonstrated that combined applications of antagonists with different modes of action that targeted different stages in the infection process could enhance target effects. Vargas *et al.* (2015) also investigated the combined effect of *Trichoderma* spp. strains and *P. lilacinum*. Although they did not observe reductions in *R. similis*, treated plants produced heavier bunches.

Summary of management measures

The different practices used for managing nematodes in bananas are summarized in [Table 17.1](#). Of paramount importance in managing nematode pests of banana is the use of healthy, nematode-free planting materials to be replanted on uninfested lands (Loos and Loos, 1960). This is practised exclusively in commercial settings, but in smallholder farming situations the adoption of sucker disinfection treatments requires greater uptake to be sufficiently effective. Although more expensive, the use of healthy plantlets is gathering pace with smallholder farmers, once the benefits have been observed and where nursery distribution networks are present, providing access to and support for farmers (Dubois *et al.*, 2006, 2013). Under permanent cultivation, nematode management opportunities are effectively limited to regular nematicide treatment; in subsistence cultivation, the only realistic or economically justifiable techniques for preventing losses from nematodes may be by applying large quantities of mulch to stimulate root growth, and by propping fruiting stems. Several of the techniques used for nematode control are also appropriate for controlling the banana weevil, which is also a widespread damaging pest. The selection of appropriate management techniques will depend largely on the local conditions and economic considerations, as well as the skill and experience of operators. There is optimism in the development and use of microbial antagonists, either as products to apply to existing crops but

especially through the enhancement of tissue culture plantlets before delivery to the farmer, while efforts in breeding for resistance are strengthened.

Methods of diagnosis

Sampling

The root systems of bananas are unlike those of short-cycle and other perennial crops, and methods for sampling need to be modified accordingly. Some of the basic principles of sampling are reviewed in Chapter 4, this volume. Suggested protocols by Speijer and De Waele (1997) and Carlier *et al.* (2002) provide excellent additional guides.

A succession of roots develops from the main corm and from the suckers until flowering; thereafter, new root growth is from the suckers only. Samples should be taken near to the base of the mother plant stem, where the highest concentration of root cortex destroyers can be found (Thomason and Caswell, 1987).

Nematode densities may differ depending on the type of root (Edmunds, 1968), while relative densities of different nematode species can occur in different roots/areas ([Fig. 17.16](#)). Root samples containing large quantities of thin, branching primary roots may contain relatively more nematodes than equivalent weights of root consisting of thicker, unbranched primaries. However, root terminology can differ between studies (Swennen *et al.*, 1986).

Table 17.1. Methods for maintaining productivity in banana plantations.

A. Established practices for decreasing nematode densities in different banana-growing systems.

1. Use of tissue-cultured (*in vitro*) plants.
 2. Rotation with alternative crops for minimum of 2 years.
 3. Fallow in the absence of banana 'volunteers' for 10–12 months.
 4. Selection of disease-free suckers.
 5. Paring diseased tissue from corms.
 6. Immersing suckers in hot water.
 7. Flooding for 8 weeks after having destroyed previous banana crop.
 8. Applying a nematicide to planting hole and in-fill soil.
 9. Regular spot applications with nematicides.
-

B. Practices that maintain productivity and vigour.

1. Support plants with bamboo poles or with string guy ropes to prevent plants toppling.
 2. Regular application of mulches of grass, leaves or organic waste (see [Fig. 17.11](#)).
 3. Grow cultivars with robust stature and wind tolerance (see [Fig. 17.4b](#)).
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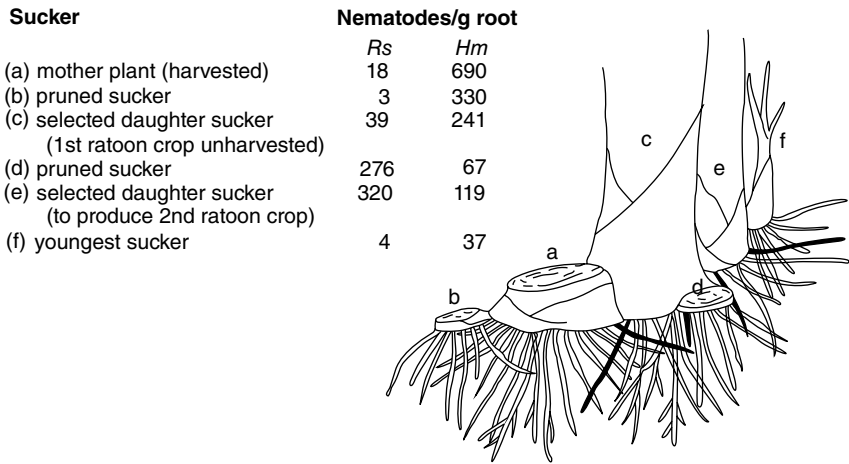


Fig. 17.16. *Radopholus similis* (*Rs*) and *Helicotylenchus multicinctus* (*Hm*) densities in the roots of the different components of a banana mat from a peaty soil, Côte d'Ivoire.

When assessing for nematodes in the field, remove root samples from close to the base of the principal pseudostem at a depth of 5–30 cm. Sampling could be monthly, quarterly, or less frequently, or at a specific stage, e.g. flowering. In detailed studies, it may be desirable to analyse separately the roots originating from suckers to different stages of development (Quénéhervé, 1990), even though this may involve the destructive sampling of entire plants (Quénéhervé and Cadet, 1986). Where *R. similis* is known to be the only important root parasite, root sampling alone will suffice, as soil densities are relatively low.

The quality of nematode data is only as good as the attention given to sampling and extraction. In summary, the basic requirements are that sufficient representative plants are sampled with consistency from where samples are taken regarding position and growth stages, within samples, and between sampling dates, and that samples are stored/transported properly before extraction to ensure survival of the nematodes. Root sampling may be best undertaken at the time of flowering.

Extraction

Ideally, samples should be processed as quickly as possible and stored properly during collection and transit (see Chapter 4, this volume). Extractions of *R. similis* and *H. multicinctus* may be affected differentially by the conditions and period of storage prior to processing (Whyte and Gowen, 1974).

With banana roots, high levels of phenolic compounds from chopped or macerated roots can cause depletion in oxygen and influence nematode recovery, which can be partly overcome by adding hydrogen peroxide to the extraction dishes (Gowen and Edmunds, 1973). Speijer and De Waele (1997) recommend extraction from a 15 g subsample of roots, cut to 1 cm size and blended for 3 × 10 s, with short intervals between each 10 s. The macerate can then be poured through nested sieves and nematodes recovered on a 38 µm sieve. It is worth following an established protocol that has been tried and tested and permits comparisons with similar studies. However, direct recovery techniques by maceration and sieving (Quimi and Villacis, 1977), maceration, sieving and centrifugation (Vilardebó, 1974) and maceration, flocculation–flotation (Escobar and Rodríguez-Kabana, 1980) are more efficient, as can be the mistifier extraction technique. Whichever extraction procedure is used, it is important to obtain a representative root sample and to use the same technique consistently.

Extraction or estimation of the sedentary endoparasites *R. reniformis* and *Meloidogyne* spp. needs to be estimated from either soil samples or, if available, by using the mistifier extraction technique.

Visual assessments

Nematode damage can be assessed physically, which will complement nematode quantification;

for example, the incidence of uprooting per hectare per month, assessments of necrosis on primary roots and corms (Bridge and Gowen, 1993; Speijer and De Waele, 1997; Fig. 17.17).

Care should be taken not to confuse lesions caused by plant parasitic nematodes with those resulting from other root-infecting pests and pathogens.

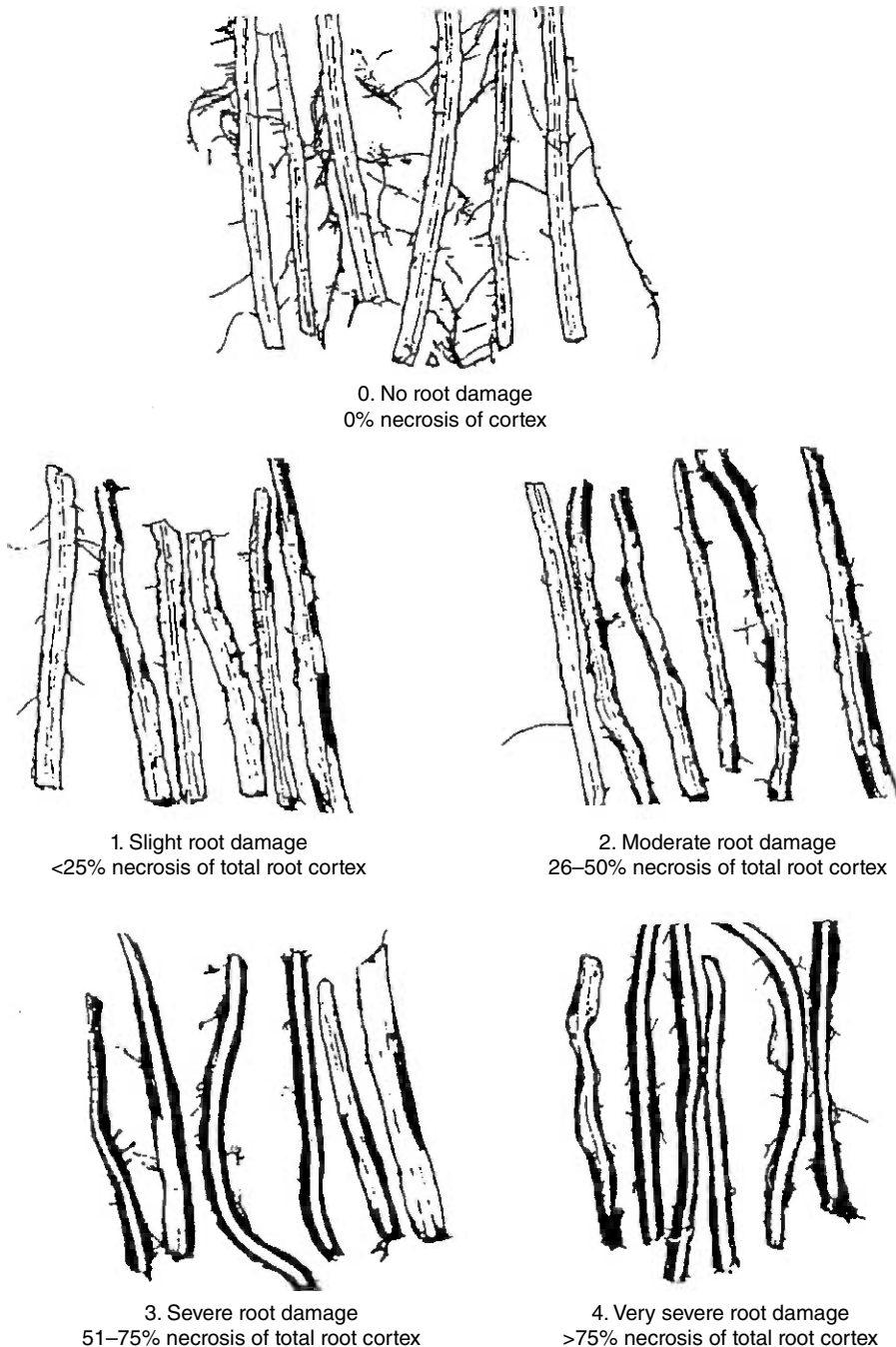


Fig. 17.17. Visual assessment of banana roots damaged by migratory endoparasitic nematodes using a root index. (From Bridge and Gowen, 1993, modified from Broadley, 1979.)

Determination of population density and crop loss

Yield loss caused by nematodes can be devastating in terms of uprooting, smaller-sized bunches and longer vegetative phase. An impaired anchorage under heavy infection levels will have obvious impact, with greater losses occurring where banana stems are not propped. Anchorage may be impaired further by the removal or suppression of suckers. Corm necrosis (and consequent root damage) may also result from weevils, which can sometimes be difficult to distinguish. During periods of low water availability, stems become weakend, with a higher incidence of snapped stems occurring in infected plants (Coyne *et al.*, 2013). Most ectoparasitic species browse only on the fine secondary and tertiary roots. Despite the often large densities recovered from soil, there are no reports of damage causing yield loss.

As banana is in a continuous state of aerial growth and root proliferation, it is difficult to determine initial inoculum potential linked to crop losses and final population densities. Despite substantial differences in population densities that can be related to crop productivity, it is probably not unreasonable to consider root infections in excess of 20/g root as a potential cause of crop losses in all commercially grown cultivars. Arguably, any infection should be considered as a threat to production over the long term.

Conclusions and Future Prospects

The global spread of Black Sigatoka, Banana bunchy-top virus, BXW and *E. oxysporum* TR4 into new banana-growing regions has had a major impact on research in phytopathology and breeding programmes, directing attention to the significance of biotic constraints. The wide variability that exists in the many different clones of both dessert and cooking bananas is now being examined aggressively for resistance and tolerance not only to these diseases but simultaneously for nematode and weevil pests (<http://breedingbetterbananas.org/>). The availability and access to tissue culture planting material now enable the free movement of genetic material internationally, both for the exchange of genetic material as well as to provide a readily available source of healthy planting material.

However, despite many years of effort, international trade is still based on the minor variants of a single genotype, *Musa* AAA subgroup Cavendish, which is heavily affected by the major diseases.

Renewed efforts in conventional banana breeding have long been voiced to introduce good agronomic qualities along with pest and disease resistance (e.g. Shepherd *et al.*, 1987; Bakry *et al.*, 1997), which has been taken seriously in the Breeding Better Bananas initiative in East Africa (<http://breedingbetterbananas.org/>), a programme focused on breeding pest and disease resistance into cooking cultivars that have a wider acceptance regionally. For the smallholder, resistance remains the only true solution to the nematode problem.

Exploitation of resistance against *R. similis* and *Pratylenchus* spp., where the latter is the dominant species, should be a major breeding priority. Other plant characters, such as root vigour that confers some tolerance to nematodes, need also to be considered. With the almost universal availability of tissue culture plants, the critical examination of pathogenicity of the different nematode species (and biotypes) on breeding lines and new cultivars can now be readily undertaken. Results collected so far, however, indicate that these objectives are quite difficult to achieve. Nematodes will continue to be a major production constraint for most types of banana. There are no major banana-growing regions in the tropics where *R. similis*, *H. multicinctus* or *Pratylenchus* spp. do not occur. *R. similis* is no doubt a debilitating constraint, especially the aggressive populations identified in Uganda, which can overcome known resistance. However, of particular concern is the shift in dominance by *P. coffeae* (and very possibly additional species) in West Africa and the increasing spread of *Pratylenchus* spp. in the Caribbean, East Africa and Asia. This needs to be monitored carefully, while establishing a strategy to deal with this. *Meloidogyne* spp. appears to be more damaging in the few special production areas outside the tropics, such as Morocco, north Yemen and Cyprus, and in Taiwan and Vietnam, while they are ominously prevalent in other areas, such as South, Central and West Africa, where they may yet prove to be a serious cause for concern.

Although significant caution has been exercised internationally around the development

of transgenic crops, banana, as a target crop for genetic modification for resistance to key pests and diseases, has attracted significant attention (Atkinson *et al.*, 2004; Shotkoski *et al.*, 2010). Among the target objectives, nematode resistance has featured strongly (Roderick *et al.*, 2012b), with successes demonstrating the potential for transgenic nematode-resistant banana in the field (Tripathi *et al.*, 2015). Should nematode resistance be simultaneously incorporated transgenically into banana with resistance to additional major diseases affecting banana markets, acceptance may change.

Refinements to nematode management in established plantations have led to a more rational use of nematicides, resulting in lower frequencies of application. The development of precision application technology in which plants are treated individually at well-defined events could also be part of a more integrated pest management (IPM) system. Access and cost discourage nematicide use in most growing systems, other than for international export. Legislative changes relating to pesticide safety have reduced the range of available nematicides. However, there is optimism for a number of biologically based nematode management options. But such options will not stand alone, nor provide universal control. Such options are subject to the vagaries of environmental variance, host suitability, nematode population, etc. They also need to be integrated into IPM packages that will facilitate their potential.

Diversity of cultivars and market opportunities

In the future, there will be a wider choice of cultivars available, particularly the new hybrids with resistance to Sigatoka diseases, such as produced by the various breeding programmes, and quite likely through transgenic techniques. There are special brands indicating the country or region of origin, and fruit with characteristic sizes, colours and/or flavours, and organic production that are now occupying 'niche markets'. Such cultivars and brands are being promoted as organic or 'Fair Trade' and represent an increasing, although relatively modest, share of the internationally traded fruit.

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18 Nematode Parasites of Sugarcane*

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Sugarcane is one of the few crops to provide commercial quantities of food, fibre and fuel. It is grown in more than 90 countries throughout the tropics and subtropics, and for some of these countries is the principal source of revenue. These countries include the Dominican Republic, Jamaica, Mauritius and Swaziland. The main product of sugarcane is, of course, sugar. In the 2013/14 season, annual world production of cane sugar exceeded 179,000,000 t, with Brazil and India being the largest producers (Table 18.1).

More than half of the cane grown in Brazil is used to produce ethyl alcohol (Schmitz *et al.*, 2003). Another potential avenue for sugarcane is the production of electricity for general use.

Sugarcane is a tall, perennial, thick-stemmed grass. It was originally proposed that modern sugarcane cultivars were complex hybrids between *Saccharum officinarum* and *Saccharum spontaneum* (Butterfield *et al.*, 2001). However, recent analysis of the chloroplast genomes of three sugarcane species have shown that modern cultivars are, in fact, a distinct species. The proposed new name for this species is *Saccharum cultum*. The centre of origin of these species is probably the South Asian mainland (Evans and Joshi, 2016).

Sugarcane plants grow in tufts or stools composed of varying numbers of stalks. At maturity,

the stalks are approximately 2–3 m in length and 20–30 mm in diameter. The stalk is composed of a series of nodes, each of which carries an axillary bud and a leaf. Carbohydrates are stored in the internodes, primarily as sucrose. Modern cultivars of sugarcane normally contain between 11 and 14% sucrose.

Cultivation

Sugarcane is propagated vegetatively by planting setts (stalk cuttings) with two or more nodes. Within a few days, roots develop from primordia around the nodes of the setts. These sett roots support the initial growth of the primary shoots that develop from auxiliary buds on the setts (Fig. 18.1). Subsequently, tillers arise, and these and primary shoots develop shoot roots that soon replace the sett roots. As the shoots grow, they compete for light and space, and a notable proportion die. Those shoots that survive increase in diameter and length as the plant grows. Depending on growing conditions, the crop is harvested after approximately 12–24 months.

Soon after harvest, new shoots emerge from auxiliary buds on the stubble and give rise to the ratoon crop. Initially the young shoots are dependent upon the roots of the previous crop

*A revision of the chapter by P. Cadet and V.W. Spaul in the second edition.

Table 18.1. Area under sugarcane in 2013 and annual sugar production in 2013/14 for the top ten sugar-producing countries in the world. (From Zhao and Li, 2015; Anon., 2017).

Country	Area under sugarcane (ha)	Production (Mt/year)	Yield (t/ha)	Sugar (Mt/year)
Brazil	9,835,000	739.27	75.17	38
India	5,060,000	341.20	67.43	27
China	1,827,000	126.14	69.03	14
Thailand	1,322,000	100.10	75.74	11
Pakistan	1,129,000	63.75	56.48	6
Mexico	783,000	61.18	78.16	6
Colombia	406,000	34.88	85.95	2.3
Indonesia	34,000	33.70	74.89	2.3
Philippines	32,000	32.00	73.49	2.5
USA	28,000	27.91	75.71	8
World	26,523,000	2165.23	81.64	175.87

Note: Mt = million tonnes.

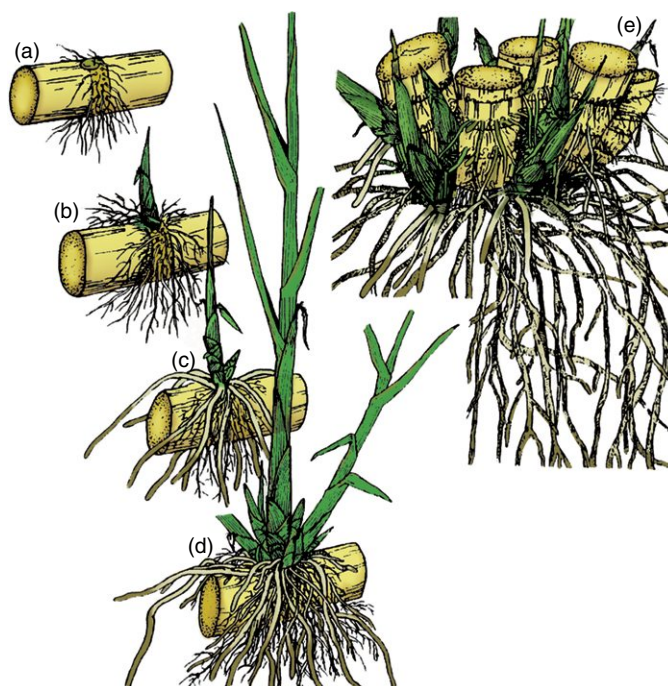


Fig. 18.1. Sequence of events in the early stages of development of plant and ratoon cane. (a) Appearance of sett roots. (b) Emergence of the bud and development of the primary shoot. Establishment of the sett root system. (c) Appearance of the shoot roots on the primary shoot and initiation of tillers. (d) Maximum density of tillers, establishment of the shoot root and disappearance of the sett root system. (e) Stool of ratoon cane showing new shoot arising from lateral buds on the stubble. Shoot roots develop at the base of the new shoots and eventually replace the stool roots (i.e. the shoot roots of the previous crop).

(stool roots) but these are replaced by new shoot roots (Fig. 18.1). The crop cycle of sugarcane is normally composed of the plant and, typically, 2–4 ratoon crops. However, the actual number

of ratoons harvested before the crop is replaced depends on growing conditions and local cultural practices. There is usually a decline in yield after the first or second ratoon crop.

Nematodes of Sugarcane

Nematode diversity in sugarcane is greater than in most other cultivated crops. More than 317 species of 48 genera of endo- and ectoparasitic nematodes have been recorded from its roots and/or rhizosphere. Certain genera are particularly widespread in cane fields, i.e. *Pratylenchus* (with at least 20 species reported from sugarcane worldwide), *Helicotylenchus* (36 spp.) and *Tylenchorhynchus* (38 spp.). Several others are common locally, e.g. *Meloidogyne* (8 spp.), *Xiphinema* (52 spp.), *Hoplolaimus* (11 spp.) and *Paratrichodorus* and *Trichodorus* (9 spp.) (Lamberti *et al.*, 1987; Spaul and Cadet, 1990; Blair *et al.*, 1999a,b; Bond *et al.*, 2000; Elbadri *et al.*, 2009; da Silva *et al.*, 2012; Wang *et al.*, 2015; van den Berg *et al.*, 2016; Bellé *et al.*, 2017).

Sugarcane is predominantly grown as a continuous monoculture with usually no more than a few months' break between removing the old ratoon crop and replanting the field. Thus, conditions tend to favour the development of relatively large populations of selected nematode species. Those most frequently cited as highly pathogenic to sugarcane are *Pratylenchus zeae*, *Meloidogyne incognita* and *Meloidogyne javanica*.

Pratylenchus

Collectively, species of *Pratylenchus* are the most common plant parasitic nematodes associated with sugarcane. Worldwide, *P. zeae* is the species most frequently encountered. A new species, *Pratylenchus parazeae* n. sp. was isolated from *Saccharum sinensis* in the Guangxi Zhuang Autonomous Region, China (Wang *et al.*, 2015).

Symptoms of damage

P. zeae causes conspicuous red, reddish-purple or brown lesions on the roots of cane (Stirling and Blair, 2000). The lesions become necrotic and turn purplish-black, causing the root system to darken in colour. This is associated with a reduction in shoot and root mass, yellowing of the leaves, stalk length reduction (Valle-Lamboy and Ayala, 1980) and reduction in stalk count (Tarte *et al.*, 1977). *P. zeae* may also adversely affect cane quality (Sujatha and Mehta, 1994) and significantly reduces the presence of fine root

hairs on sugarcane roots (Blair, 2005). *Pratylenchus brachyurus* has been shown to cause damage to the vascular system and cause destruction of cortical cells, without affecting root or shoot mass (Onapitan and Amosu, 1982). In contrast, Koike and Roman (1970) reported that *P. brachyurus* affected the length and mass of stalks, with no evident damage symptoms to the roots. *P. parazea* n. sp. causes reddish, brown to dark lesions along the entire roots, except for the root tips. The above-ground parts of the plant become stunted and yellow. Nematode infection also results in massive necrosis, granular cytoplasm and hypertrophied nuclei of cortical cells (Wang *et al.*, 2015). The economic importance of this nematode species is not clearly understood; however, this nematode is often isolated along with *P. zeae* from the same field.

Environmental factors affecting parasitism and pathogenicity

In Australia and South Africa, *Pratylenchus* spp. are widespread in all soils (Spaul, 1981; Blair *et al.*, 1999a,b; Blair, 2005), but higher numbers and higher frequency have been reported in clay soils in comparison to sandy soils in West Africa, Taiwan, Louisiana in the USA and the southern states of India (Hu *et al.*, 1968; Cadet, 1987; Mehta, 1992; Bond *et al.*, 2000). In Martinique, the spatial distribution of *P. zeae* was shown to be homogeneous in a soil with a normal A horizon, but had concentrated along the cane rows where this horizon had been removed by levelling (Delaville *et al.*, 1996).

P. zeae has shown preferences for different root types, but numbers in the soil were similar (Blair *et al.*, 1999a). In Burkina Faso and South Africa, they have been found to be more numerous in the sett roots than in the shoot roots of plant cane (Cadet and Spaul, 1985). In South Africa, more individuals were recovered from the shoot roots than from the stool roots of ratoon cane (Spaul and Cadet, 1991). In Australia, densities were greater in the roots of plant cane than in those of ratoon cane (Blair *et al.*, 1999a). Penetration of roots by *P. zeae* was greater at higher temperatures (26–28°C), irrespective of soil type (Mehta and Sundararaj, 1990).

Large differences were found in the suitability of different cane cultivars as hosts to *P. zeae*, with many more individuals being recovered from the roots of some cultivars than others

(Dinardo-Miranda, 1994; Mehta and Somasekhara, 1998; Blair *et al.*, 1999a). It is proposed that resistant cultivars produce a hypersensitive response and thicken cell walls in response to *Pratylenchus* infection (Kathiresan and Mehta, 2002a), and also decrease catalase activity (Kathiresan and Mehta, 2003). Research has also demonstrated that the levels of phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in roots and leaves are greater in resistant clones compared to susceptible sugarcane clones (Kathiresan and Mehta, 2005).

Disease complexes

The pathogenicity of *P. zeae* to sugarcane is affected by other organisms. In combination with *Pythium graminicola*, *M. incognita*, or both these organisms simultaneously, *P. zeae* had significantly less effect on the mass of cane roots than in their absence (Valle-Lamboy and Ayala, 1980). Necrosis of the roots was more than halved when one or both of the other pathogens were present. When *P. zeae* and *M. incognita* are both present, an increase in the initial population of one of the genera negatively affects the reproductive capacity of the other genus. In pot trials, co-infection by the nematodes did not show an additive effect on the vegetative growth of the

sugarcane compared to a single genus (Fontana *et al.*, 2015). In addition, Rashid and Singh (2002) showed that the incidence of sugarcane wilt caused by fungi *Fusarium sacchari* and *Acremonium implicatum* increased in the presence of plant parasitic nematodes. It was hypothesized that the nematodes provided points of entry for the fungi.

Economic importance

Damage thresholds are not well defined for nematode infections because extraction methods affect nematode counts and environmental factors affect the response to control measures. However, Stirling and Blair (2000) have proposed thresholds that may result in a significant reduction in cane yield. Glasshouse and field trials suggest that numbers in excess of 600/kg of dry soil cause reduced sugarcane growth (Table 18.2). These levels are exceeded in many fields in Australia; thus, *P. zeae* is considered the primary nematode pathogen of sugarcane in that country (Blair *et al.*, 1999b). *P. zeae* is also of particular importance in Panama (Pinochet, 1987), Burkina Faso and South Africa (Cadet and Spaull, 1985), the USA (Birchfield, 1984) and in the Brazilian state of Pernambuco (de Moura *et al.*, 1999). Barbosa *et al.* (2013) showed that

Table 18.2. Nematode densities considered responsible for reduced sugarcane growth. (From Blair, 2005.)

Nematode (s)	Nematodes/g root (dry wt.)	Nematodes/kg soil (dry weight)	Experimental source and reference
<i>Pratylenchus zeae</i>		>1000	glasshouse (Nath <i>et al.</i> , 1975)
<i>Paratrichodorus minor</i>		>1000	glasshouse (Apt and Koike, 1962b)
<i>Helicotylenchus dihystra</i>		>6500	glasshouse (Apt and Koike, 1962a)
<i>Pratylenchus zeae</i>		>600	field shoot roots (Bull, 1979)
<i>Pratylenchus zeae</i>	3000		field sett roots (Cadet and Spaull, 1985)
+			
<i>Meloidogyne</i> spp.	750		field shoot roots (Chandler, 1978)
<i>Pratylenchus zeae</i>			
+			
<i>Meloidogyne</i> spp.			
+			
<i>Achlysiella williamsi</i>	>500		
<i>Pratylenchus zeae</i>			field ratoon roots (Spaull and Donaldson, 1983)
+			
<i>Meloidogyne</i> spp.	1000		
<i>Trichodorus</i> spp.		100–200	field shoot roots (Cadet and Spaull, 1985)
+			
<i>Xiphinema</i> spp.		400–800	

both *P. zae* and *P. brachyurus* significantly reduced the fresh weight of the aerial parts of the plant and millable stalks compared to the uninoculated control of the Brazilian CTC 2 cultivar. *P. brachyurus* also reduced the internode length of the stalk, thereby indicating stress. In this study, it was concluded that although *P. zae* had a greater biotic potential than *P. brachyurus*, *P. brachyurus* was more aggressive as it required 1000 times less individuals than *P. zae* to cause similar damage.

Meloidogyne

M. incognita and *M. javanica* have been found in many sugarcane areas, and most of the numerous records of unidentified *Meloidogyne* probably refer to one or both of these species. Seven other species have been identified from cane: *Meloidogyne acrita*, *Meloidogyne arenaria*, *Meloidogyne hispanica*, *Meloidogyne kikuyensis*, *Meloidogyne thamesi*, *Meloidogyne enterolobii* and *Meloidogyne ethiopica*, but none is widespread. *M. enterolobii* was reported on sugarcane in Brazil, but all screened cultivars were reported as poor hosts or immune to infection (da Silva *et al.*, 2012). Conversely, *M. ethiopica* was shown to be highly pathogenic on the variety RB72454, showing a 60% reduction in plant height and a reproduction factor of 16.6 (Bellé *et al.*, 2017).

Symptoms of damage

The symptoms of damage are distinct but are usually diagnosed less easily than in many other susceptible crops. Galls formed by *M. incognita* and *M. javanica* develop on the tips of the sett roots and young shoot roots (Fig. 18.2). In contrast, galls of *M. kikuyensis* form at 90° to the root, resembling a nitrogen-fixing nodule (Eisenback and Dodge, 2012). The galls are often small and discrete and not easily detected, except in young plant cane. Williams (1969) illustrated elongated swellings on the tips of sugarcane roots and the proliferation of lateral roots immediately proximal to the gall. In old suberized roots, females may develop at various positions along the root without inducing galling (Martin, 1967). In pot-based experiments, *M. incognita* and *M. javanica* reduced the top weight and root weight of sugarcane (Valle-Lamboy and Ayala,



Fig. 18.2. Terminal galls on sugarcane plants infected with *Meloidogyne* spp.

1980; Novaretti, 1981). Species of *Meloidogyne* may also reduce the number of tillers developed by sugarcane (Salawu, 1986).

Environmental factors affecting parasitism and pathogenicity

Species of *Meloidogyne* are found more frequently in sandy soils than in finer textured soils (Spaull, 1981; Blair *et al.*, 1999a,b). Greater populations of *M. incognita* and *M. javanica* are recorded in sett roots than in shoot roots of plant cane (Cadet and Spaull, 1985).

Populations of root knot nematode may be influenced by the presence of phytopathogenic fungi. Thus, far fewer *M. javanica* were recorded from the roots of sugarcane infected with the seedling blight fungus *Curvularia lunata* than from uninfected plants (Khurana and Singh, 1971). Conversely, the presence of other pathogens favoured colonization of sugarcane roots by *M. incognita*, with more galls being produced in the presence than in the absence of *P. graminicola*. When *P. zae* was also present, even more galls developed, although in both cases the size of the galls was smaller than normal (Valle-Lamboy and Ayala, 1980).

Reproduction of *M. incognita* was reduced significantly under water stress conditions. Furthermore, nematode inoculation did not affect cultivar response to drought stress (Santos *et al.*, 2013; Quintela *et al.*, 2015).

Disease complexes

The effect of *M. javanica* and *C. lunata* on sugarcane was greater when the two organisms were inoculated together than when either was inoculated

alone (Khurana and Singh, 1971). A similar interaction was recorded between *M. incognita* and *P. graminicola* on sugarcane seedlings (Apt and Koike, 1962a). However, in another study, the combination of *M. incognita* and *P. graminicola*, or *M. incognita* and *P. zeae*, or all three species together, had significantly less effect on root mass of sugarcane than when either of the nematodes was acting alone (Valle-Lamboy and Ayala, 1980).

The effect of the combination of *M. incognita* race 1 and ratoon stunting disease (*Leifsonia xyli* subsp. *xyli*) on sugarcane in pots was additive rather than synergistic (Regis and de Moura, 1989).

Economic importance

The same limitations on the use of damage thresholds given for *Pratylenchus* apply to species of *Meloidogyne*. As with *Pratylenchus*, thresholds for *M. javanica* have also been proposed (Stirling and Blair, 2000). In combination with *P. zeae*, numbers in excess of 750 nematodes/g of dry root weight will result in reduced sugarcane growth (Table 18.2).

Together with *P. zeae*, *M. incognita* and *M. javanica* are probably the most important parasitic nematodes of sugarcane worldwide. Estimates of crop loss due to species of *Meloidogyne* in Mexico, Central and South America, the Caribbean and South-east Asia ranged from 6 to 9%, although these were not supported by experimental data (Sasser, 1979). Cadet and Spaul (2003) found that a 30% reduction in yield between two field trials situated 800 m apart on similar sandy soil was due to the presence of *M. javanica* in one trial but not the other. Modelling the yield data over a plant crop and four ratoons revealed that where *M. javanica* did not occur, cane could have been grown for up to 4 years longer before it had to be replanted.

Nematode communities

Attention has so far focused on species of *Pratylenchus* and *Meloidogyne*, as they are widespread on sugarcane and generally considered the most damaging plant parasitic nematodes. However, these and other nematodes associated with sugarcane rarely occur alone in the soil but are present in communities comprising a number of

species. Surveys from several countries showed that the number of genera present in a single soil sample ranged from 1 to 12, with an average of between 3.2 and 7.9 (Lamberti *et al.*, 1987; Spaul and Cadet, 1990; Blair *et al.*, 1999a,b; Bond *et al.*, 2000; Shahzad *et al.*, 2010; Bellé *et al.*, 2014; Ramouthar, 2014). All genera within the community have different levels of distribution and pathogenicity on sugarcane, and thus differing effects on sugarcane yield. This community also includes free-living nematodes that have a beneficial effect on soil health, and thus on sugarcane growth.

Long-term sugarcane production alters the nematode community such that soils contain higher numbers of plant parasitic nematodes as opposed to free-living or beneficial nematodes (Cardoso *et al.*, 2012, 2015; Arieira *et al.*, 2013). Much of the research in recent years has focused on understanding the impact of different farming practices in relation to nematode communities, particularly with regard to its impact on sugarcane yield.

Symptoms of damage

The symptoms of nematode damage on the roots of sugarcane, such as reduction in root mass and fine root hairs (Fig. 18.3), are not unlike those observed on other crops. The symptoms listed under the genera in Table 18.3 were recorded from pot cultures of single species. However, in field-grown cane, roots show the combined damage symptoms of all the nematodes, and other soil-borne diseases infecting the root system. Since several species cause similar damage, it is usually not possible to identify the



Fig. 18.3. Root biomass of nematicide untreated (left) and nematicide treated (right) plots.

Table 18.3. Symptoms of damage caused by different nematode genera on sugarcane in pots and by communities of nematodes in sugarcane fields. (From Cadet and Spaull, 2003.)

	<i>Pratylenchus</i> ^a	<i>Meloidogyne</i>	<i>Helicotylenchus</i>	<i>Tylenchorhynchus</i>	<i>Paratrichodorus</i>	<i>Xiphinema</i>	<i>Hoplolaimus</i>	Community of nematodes in the field ^b
Reduction in shoot and root mass	+	+	+	+	+	+	+	+
Reduction in number of shoots	+	+						+
Necrosis of cells in root cortex	+		+	+	+	+	+	+
Red/purple/brown/ pink lesions on roots	+		+				+	+
Fewer roots/sparse root system	+		+		+	+	+	+
Stunted roots			+	+	+	+		+
Distorted roots	+		+					+
Galls		+				+		+
Blackening of the roots	+							+
Reduction of fine root hairs								

Notes: Not all the symptoms are observed in all situations.

^aColumns 2–8: summary of data from Spaull and Cadet (1990). ^bColumn 9: unpublished observations on symptoms found in sugarcane fields in: Australia (G.R. Stirling, Queensland, Australia, 2003, personal communication), Brazil (W.R.T. Novaretti, Brazil, 2003, personal communication), Burkina Faso (P. Cadet, unpublished data), Côte d'Ivoire (P. Quénehervé, Martinique, 2003, personal communication) and South Africa (V.W. Spaull, unpublished data; Blair, 2005.).

nematodes responsible by the symptoms alone. In addition, the coloured lesions on the roots associated with the feeding of *P. zeae* and species of *Hoplolaimus* are similar to the early symptoms produced by root-rotting fungi such as *Pythium arrhenomanes* and *Pachymetra chaunorhiza* (Croft and Magarey, 2000; Hoy, 2000). The situation is also complicated by the natural darkening of the epidermis as the root suberizes.

In India, chlorosis of the leaves is commonly attributed to nematode damage (Mehta, 1992). There are also some above-ground symptoms that are often associated with the damage caused by nematodes. These are reduced shoot height and number (Fig. 18.4) and slow canopy development. The latter results in a more open appearance of the cane (Fig. 18.5), while the leaves curl longitudinally and appear spiky. These are, however, also symptoms of drought-stressed cane and thus cannot be used for diagnosis.

Due to the myriad of organisms that interact and occur within the soil rhizosphere, as well as nutrient imbalances that may affect the

growth of roots, it is very difficult to diagnose nematode damage accurately. Similar root damage can also be caused by microorganisms (Croft and Magarey, 2000; Hoy, 2000) and the larvae and adults of some insects (Wilson, 1969). High levels of soil aluminium (Al), low levels of phosphorus (Humbert, 1968) and soil compaction or poor aeration can also lead to root damage. Only *Meloidogyne* can be diagnosed with confidence because the female may be observed in galls by dissecting the root (Fig. 18.6). In other cases, it may not be prudent to link so-called typical symptoms with one or other species of nematode, as nematodes are only one component of a complex of factors that affect root growth.

Abiotic soil factors affecting parasitism and pathogenicity

Nematode community composition varies widely from country to country, from one soil type to the next, and even over short distances within a field. Communities in sandy soils are more likely to include larger populations of species of *Meloidogyne*, *Hoplolaimus*, *Trichodorus* and/or *Paratrichodorus* than those in the finer textured soils (Spaull, 1981; Mehta, 1992; Blair *et al.*, 1999a,b; Bond *et al.*, 2000). In South Africa, the distribution of communities containing larger populations of *Meloidogyne* was restricted more by soil type than by climatic or topographic factors (Spaull *et al.*, 2003). In contrast, the distribution of species of *Pratylenchus* and *Helicotylenchus* often appears to be unrelated to soil texture (Spaull, 1981; Blair *et al.*, 1999a), although reports from the USA and India indicate that *Pratylenchus* is more numerous in clay soils (Hall and Irey, 1992; Mehta, 1992). *Xiphinema mampara* was found more frequently in clay loams and clays, whereas *Xiphinema elongatum* tended to prefer the sandy soils (Spaull and Heyns, 1991). Greater numbers of *Meloidogyne*, *Hemicycliophora*, *Hoplolaimus* and *Paratrichodorus* were recorded in sandy soils compared with soils with high levels of organic matter (Hall and Irey, 1992).

Soil texture seemingly has the greatest influence on, or is the factor most correlated to, the pathogenicity of the nematode community. The effect of nematodes on sugarcane decreases with increasing clay content. In Burkina Faso and Côte d'Ivoire, there is no significant response to treatment with a nematicide in ratoon cane,



Fig. 18.4. Difference in shoot count and height of nematicide untreated (left) and nematicide treated (right) plots.



Fig. 18.5. Open rows of sugarcane due to nematode infection. The better rows surrounding the plot have been treated with a nematicide.

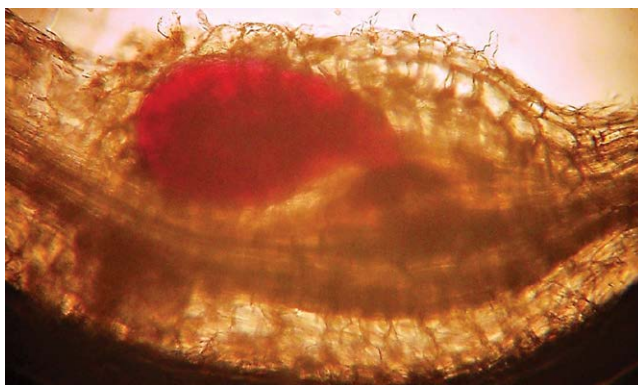


Fig. 18.6. Swollen *Meloidogyne incognita* female attached to sugarcane root. (Photograph courtesy of A. Chaves.)

irrespective of soil type. The effect of soil texture on pathogenicity is due partly to the ease of movement of nematodes in sandy soils. In plant cane in West Africa, the invasion of the sett roots by endoparasites was much more rapid in the coarser textured soils. The consequent damage to these roots delayed and disrupted the normal tillering process, with the result that the cane

developed fewer stalks (Cadet *et al.*, 1982). In finer textured soil, the endoparasites invaded the sett roots more slowly and caused less damage during the tillering phase. However, the main reason that nematodes have a greater impact in sandy soils is that they have much lower water-holding capacities than heavy textured soils. Plant parasitic nematodes feed on and limit the

growth of roots of cane, but the effect of the restricted root system on the uptake of water will be greater in a soil with a low water-holding capacity (Wallace, 1973).

An association between soil parameters and nematode genera has also been described by Rimé *et al.* (2003). They found a negative correlation between *P. zeae* and organic matter, total N and Fe levels in the soil. In contrast, *Helicotylenchus dihystrera* showed a positive correlation with these variables. This inverse proportionality was noted for pH as well, with *Pratylenchus* showing a positive correlation and *Helicotylenchus* a negative one. In addition, *Helicotylenchus* was correlated negatively with calcium and magnesium and correlated positively with Al.

Nematode community structure may also be influenced by both altitude and temperature. In South Africa, communities with larger populations of *P. zeae* and *X. elongatum* tended to occur at altitudes below 300 m where average annual temperatures exceeded 20°C. The reverse was true for communities with larger numbers of *H. dihystrera* and a species of *Rotylenchus* (Spaull *et al.*, 2003). In Mauritius, *X. elongatum* was largely confined to altitudes below 250 m where rainfall was less than 2000 mm/year. It was less commonly found in the central, more elevated part of the island, where rainfall was greater and *Xiphinema krugi* was widespread (Williams and Luc, 1977; Lamberti *et al.*, 1987). Similarly, *Xiphinema americanum* s.l. was not found in sugarcane fields in Hawaii above an altitude of about 230 m (Anon., 1961).

Biotic factors affecting parasitism and pathogenicity

The main biotic factor affecting nematode pathogenicity is the plant itself. There are large differences in the suitability of different cultivars as hosts to certain species (Dinardo-Miranda, 1994; Mehta *et al.*, 1994a; da Silva *et al.*, 2012; Barbosa *et al.*, 2013). Stress induced by weeds and viruses may also affect the capacity of nematodes to multiply on sugarcane (Showler *et al.*, 1990).

Two other important biotic factors affecting nematode community are the crop stage of the sugarcane and the agronomic practices during cultivation. In most countries, cane is cropped over a number of ratoons before the crop

is destroyed and the field replanted. During this period, the soil remains largely undisturbed, and the balance between the nematode populations within the community may change. In Burkina Faso, over a period of five crops, the proportion of *Hoplolaimus* in the roots increased, while a decline in the proportions of *Meloidogyne* and, especially, *Pratylenchus* was noted (Cadet, 1985). Overall, there was an increase in ectoparasites from the first to the fourth ratoon. Similarly, in the USA, the size of the nematode community was greater in ratoon crops than in the plant crop (Bond *et al.*, 2000). Ramouthar *et al.* (2012) also showed an increase in ectoparasites over a plant crop and six ratoons. However, in Australia, densities of *P. zeae* were greater in the plant crop than in the first ratoon, and there was no consistent effect of crop stage on the other species (Blair *et al.*, 1999a).

The composition of the community also affects parasitism. Certain species interfere with each other to the extent that some coexist less frequently than others (Cadet and Debouzie, 1990; Sujatha and Mehta, 1993, 1995). For example, Sujatha and Mehta (1997) observed that concomitant inoculation of *M. javanica* and *P. zeae* caused less crop loss than when each species was cultured in isolation. The pathogenicity of a nematode community to sugarcane may be reduced when *H. dihystrera* is the dominant ectoparasite. Cadet *et al.* (2002) found that significantly greater yields were recorded from plots that had a higher proportion of *H. dihystrera*, relative to the other ectoparasites, and a lower proportion of *M. javanica*, relative to the other endoparasites. A similar association of *H. dihystrera* with better yielding cane was reported in Burkina Faso by Cadet (1986). Analysis of two sites in close proximity on the north coast of KwaZulu Natal (South Africa) showed that both sites had similar nematode numbers, but one site was dominated by *H. dihystrera*. This site had higher-than-average yields for the soil type in that area (Rimé *et al.*, 2003). In addition, harvest procedures that resulted in an above-ground stubble altered the nematode community to one dominated by *H. dihystrera* instead of *X. elongatum* (Berry *et al.*, 2007b).

Free-living nematodes have been associated with nematode-suppressive soils, and this can be influenced by agronomic practices. An increase in the organic matter of soils increases the number of microorganisms associated with the soil. This,

in turn, increases the free-living nematode numbers, within which predators are contained. In addition, microorganisms that parasitize nematodes are also enhanced within the soil. Stirling *et al.* (2010a) found that despite the higher proportion of the roots in the top 2 cm under a trash blanket, a low number of plant parasitic and a higher number of free-living nematodes were found. This suppression was possibly due to the high soil organic matter content. Crop rotation, reduced tillage, controlled traffic and residue retention have been shown to increase the number of free-living nematodes, and in particular fungivorous nematodes (Stirling *et al.*, 2010b). In contrast, Mondino *et al.* (2010) found no significant difference in taxonomic diversity and disturbance indices between conventional and reduced tillage systems in a long-term study in Brazil.

Nematode–sugarcane interaction

The nematode–sugarcane interaction is a complex phenomenon. The damage to sugarcane roots may be caused simultaneously by a number of nematode species and other soil-borne pathogens. Moreover, the nematode–plant interaction is complicated by the fact that one root system is replaced by another during the growth of the crop. Interactions of soil-borne pathogens and inherent cultivar resistance/tolerance all affect nematode population dynamics. To understand the nematode interaction with the sugarcane plant, it is necessary to consider the different components of the nematode community in relation to both the development of the roots and the evolution of those plant parameters that contribute to yield. Sugarcane yield is a function of the number, length and diameter of the stalks. Root damage by nematodes causes a reduction in the number and length of stalks, and sometimes influences stalk diameter and sucrose content.

Plant cane

In Africa, Cadet and Spaull (2005) have reported that in plant cane, the reduction in the number of stalks takes place primarily during the period of maximum tiller development, i.e. while the cane plant is largely dependent on the sett root system. A reduction in the length of stalks may also be apparent at this time, and in the presence

of certain nematode communities, this increases in magnitude through to harvest. Stalk length may thus be affected by damage to both the sett and the shoot roots. These observations are in agreement with those of Blair and Stirling (2007) in Australia. These authors found that in plant cane, *P. zeae* was the most prevalent nematode species, followed by *M. javanica*. Ectoparasites such as *H. dihystra*, *Tylenchorhynchus annulatus*, *Nanidorus minor*, *Criconea* spp. and *X. elongatum* were also present in high numbers in some soils. The number of *P. zeae* and *M. javanica* peaked at mid-season and declined towards harvest, due to the reduced number of fresh roots towards the end of the growing season. The damaging effects of nematodes were manifested through the effects on stalk elongation rather than on stalk establishment.

When Cadet and Spaull (2005) compared the results from field trials in Burkina Faso and South Africa, they showed that in Burkina Faso, crop loss in plant cane was due more to a reduction in the number of stalks than to a reduction in the length of the stalks. The reverse was true for South Africa (Fig. 18.7). The authors related the patterns of change in the nematode populations to the patterns of change in the development of the sugarcane crop.

1. In both Burkina Faso and South Africa, damage to the sett roots by large numbers of *Meloidogyne* and *Pratylenchus* delayed the emergence and retarded the development of many of the primary shoots, which either produced fewer tillers or were unable to compete successfully with those that developed more rapidly.
2. The suppression of tillering was greater in Burkina Faso than in South Africa because, in the former locality, there was a much greater rate of invasion of the sett roots by endoparasites.
3. *Xiphinema*, and probably *Trichodorus* and *Paratrichodorus*, caused extensive damage to the shoot roots in South Africa, which restricted water uptake and thus limited stalk elongation.
4. The dominant ectoparasite in Burkina Faso, *H. dihystra*, had little effect on sugarcane compared with species of *Xiphinema* and trichodorids.
5. Although nematodes caused some damage to the shoot roots in Burkina Faso, this had less effect on water uptake and thus on stalk elongation than in South Africa, because the cane was irrigated.

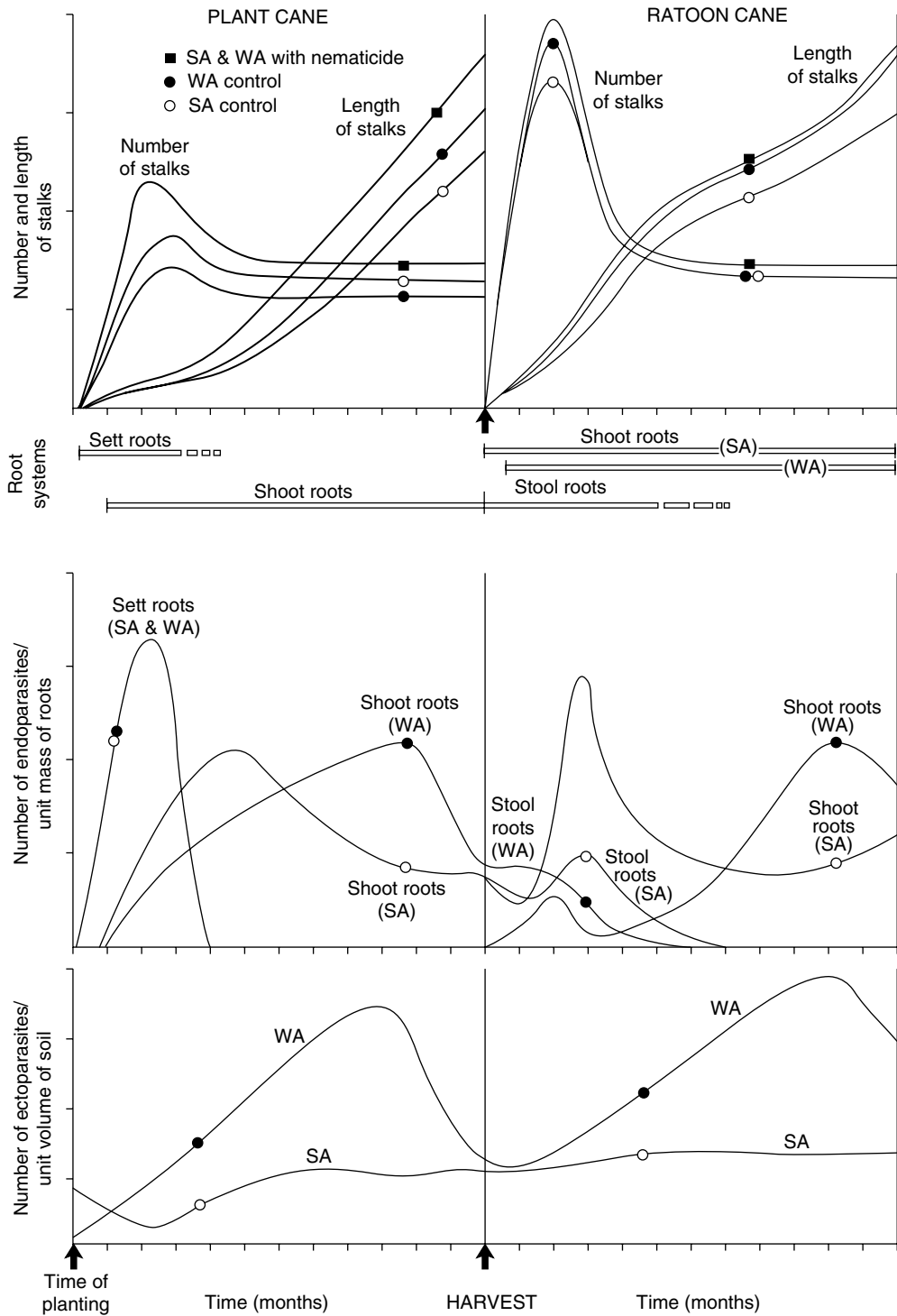


Fig. 18.7. Pictorial representation of the patterns of change in the numbers of nematodes in relation to the patterns of change in the development of sugarcane in South and West Africa.

Ratoon cane

Plant parasitic nematodes have variable effects on ratoon crops. Very little effects have been shown in Burkino Faso, whereas significant impacts were observed in Brazil, Australia and South Africa (Cadet and Spaull, 2005; Blair and Stirling, 2007) (Table 18.4). As was done for the plant crop, an attempt was made to understand the relationship between nematodes and ratoon cane by monitoring the nematode populations and the development of the cane in Burkina Faso and South Africa (Spaull and Cadet, 1991). It was deduced that the lesser root invasion in Burkino Faso was due to the very low number of endoparasites, whereas damage caused by the ectoparasites *Xiphinema* and *Paratrichodorus* to the roots in South Africa had impacted on stalk elongation.

In Australia, Blair and Stirling (2007) found that ratoon tillering (stalk number or length) was not related to the total number of nematodes at the early stage of growth. This is possibly because buds that produce new shoots are initially dependent on reserves from the stump, and therefore are not vulnerable to root pathogens. By mid-season, an increase in stalk length was correlated to the number of endoparasites (*P. zeae* and *Meloidogyne* spp.), and when both stalk length and stalk numbers were considered, the increase was shown to relate to the total number of nematodes (endoparasites + ectoparasites) (Fig. 18.8). In the ratoon, the number of endoparasites controlled in the soil was correlated significantly with increases in final yield. Nevertheless, for the ratoon crop, ectoparasites were partly responsible for the decrease in stalk length. It was concluded that in older crops, the whole community of nematodes

might need to be considered when assessing the impact of plant parasitic nematodes on ratoon crop growth.

Nematode communities and disease complexes

In addition to the interaction of sugarcane roots with nematodes as discussed earlier, a broader disease complex may also occur in the rhizosphere. Spaull and Bailey (1993) reported that the combined effect of disease and nematodes on sugarcane was additive rather than synergistic. In India, Mehta (1992) found the association of *Pratylenchus*, *Hoplolaimus* and *Tylenchorhynchus* with *Fusarium* and *Acremonium* in the wilt disease complex. In Australia, Bhuiyan *et al.* (2016a) found a negative correlation between the ectoparasite *H. dihystrera* and the endoparasite *M. javanica*, and a positive correlation between *M. javanica* and *P. minor* (Table 18.5) in a 3-year trial. A differential interaction was observed between nematodes species and *P. chaunorhiza*, a root-rotting oomycete. *P. chaunorhiza* causes serious damage to sugarcane roots in Australia, and is present in almost all sugarcane fields. There was a negative correlation between *Pachymetra* spore numbers and both *P. zeae* and total nematode numbers, and a positive correlation between *H. dihystrera* numbers and *P. chaunorhiza* spore counts. Although the interaction between *H. dihystrera* and *P. chaunorhiza* is not clear, it can be deduced that the ectoparasite *H. dihystrera* lives outside of sugarcane roots and feeds on the outer epidermis of the root, which creates a wound. The wound created by *H. dihystrera* may act as an avenue for *P. chaunorhiza* infection. On the other hand, *P. zeae* is an endoparasite, lives within the root tissue and is the most abundant nematode

Table 18.4. Yield response in per cent to treatment with nematicide in four countries.

	Australia1	Australia2	Brazil	Burkina Faso and Côte d'Ivoire	South Africa
Plant crop	23	16	29	67	14
First ratoon	12	11	16	9	14
Second ratoon	20	16	15	0	15
Third ratoon	–	–	11	7	7

Notes: Data for Australia1 are from 15 trials in plant cane and two trials in ratoon (Stirling and Blair, 2001); Australia2 are from 14 trials in plant cane, 12 trials in first ratoon and three trials in the second ratoon (Blair and Stirling, 2007); Brazil, one trial (Novaretti, 1992); Burkina Faso and Côte d'Ivoire, 16 trials (P. Cadet, unpublished data); and South Africa, 15 trials for the plant crop and first ratoon and 14 trials for the second and third ratoons (SA Sugarcane Research Institute, unpublished data).

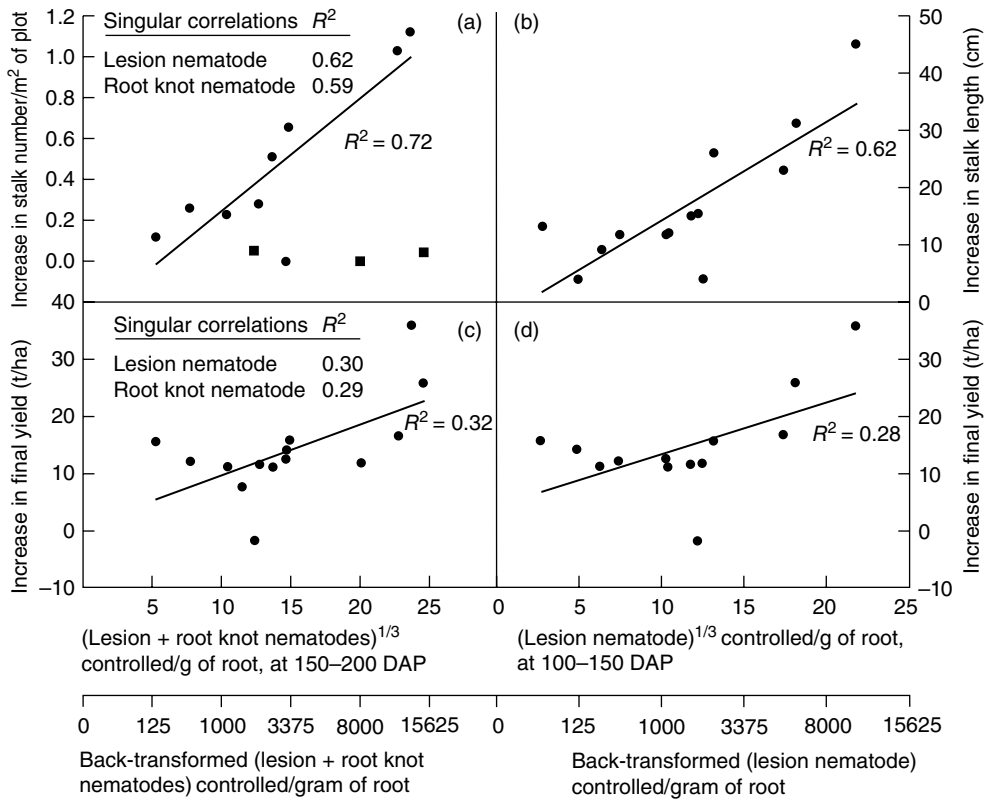


Fig. 18.8. Relationship between (a) stalk length by number, (b) stalk length and (c, d) final yield responses in ratoon crops following nematicide treatment and the number of nematodes controlled by the nematicides. (From Blair and Stirling, 2007.)

Table 18.5. Pearson correlation coefficients to compare *Pachymetra* spore counts/kg of soil and nematode counts/200 g of soil. (From Bhuiyan *et al.*, 2016a.)

	<i>Pratylenchus</i> <i>zeae</i>	<i>Meloidogyne</i> <i>javanica</i>	<i>Helicotylenchus</i> <i>dihystera</i>	<i>Paratrichodorus</i> <i>minor</i>	Total nematodes
<i>Pachymetra chaunorhiza</i>	−0.53***	−0.09ns	0.32**	−0.12ns	−0.36**
<i>Pratylenchus zeae</i>		0.016ns	−0.16ns	0.06ns	–
<i>Meloidogyne javanica</i>	–	–	−0.3**	0.24*	–
<i>Helicotylenchus dihystera</i>	–	–	–	−0.21ns	–

Notes: Data from Australia, in 3 years of trials from plant, first and second ratoon crop. ***, ** and * = significant at 0.0001, 0.001 and 0.05 levels, respectively; ns = not significant.

in sugarcane soils. *P. chaunorhiza* and *P. zeae* would compete for space and food source within root tissue. The competition between them may also be influenced by the root mass of the cane. Cane severely affected by *P. chaunorhiza* may provide reduced root mass for nematode breeding.

There has been significant research conducted to understand the interaction of nematodes and fungal pathogens in other crops. Back *et al.* (2002) documented four mechanisms of synergistic interactions between pathogenic fungi and nematodes: (i) utilization of nematode-induced

wounds for fungal infection; (ii) nematode-induced physiological change that makes plants vulnerable to fungal pathogens, or vice versa; (iii) modification in rhizosphere environments due to nematode infection in roots attracts fungal pathogens; and (iv) reduction of host resistance due to nematode or fungal infection.

Control measures

Nematodes have been considered as a yield-limiting factor in sugarcane in many countries like Australia, South Africa, Burkina Faso, China, Japan and Brazil. Due to the subtle effect of nematodes on the crop and inadequate survey data, nematodes in most of the sugarcane-growing countries are not well understood. In Australia, a comprehensive survey by Blair and Stirling (2007) found high numbers of nematodes in all soil types, including high clay soils. Control measures are thus justified where the nematode population is high.

Cultural practices

Unlike other crops, cultural practices to control sugarcane nematodes have not been widely practised. In South Africa, mixing the sandy topsoil with clay subsoil and the irrigation of sandy soil had maintained the nematode population under control and increased crop production (Cadet and Spaull, 2005). The time of planting may also influence the effect of nematodes on sugarcane. In Brazil, the best yield was obtained when planted in March (autumn) compared to that in December, January, February or April. In Taiwan, spring-planted crops were found to be less tolerant to nematodes compared to autumn-planted crops (Cadet and Spaull, 2005). In Australia, long-term field trials as part of the Sugar Yield Decline Joint Venture programme (SYDJV) found that minimum tillage reduced production costs and improved soil health (Garside *et al.*, 2004; Braunack and McGarry, 2006). Stirling (2008) demonstrated that nematode multiplication in undisturbed or minimum till soil was significantly lower compared to disturbed (tillage, fumigation, autoclaved or long bare fallow) soil. When comparing sugarcane planting by conventional tillage or by direct drilling (minimum till), populations of lesion nematodes

(*Pratylenchus* spp.) were initially reduced by conventional tillage. However, they resurged rapidly to reach higher numbers compared to drill planting at the end of the plant crop. This tillage effect was observed with *M. javanica* and *X. elongatum*, as populations of both nematodes were significantly higher following conventional tillage (Stirling *et al.*, 2011a). It was evident from several field trials that populations of plant parasitic nematodes declined when soils were mulched with a trash blanket and were not disturbed by tillage.

Fallowing, intercropping and crop rotation

Sugarcane is grown as a monoculture and there is a relatively short period between eliminating the previous crop, plough-out and planting a new crop. This means that sugarcane-specific pests and pathogens that are present at the end of one crop are simply carried over to the following crop. In Australia, Blair *et al.* (1999a) found that fallowing had a short-term effect on nematode populations, which was evidenced by the high number of *P. zeae*, *Meloidogyne* spp., *T. annulatus* and *H. dihystra* on the plant crops. It was clear that the short period of 2–10 months did not affect the nematode population, as they were quite likely to survive on weed hosts. Also, the fresh root growth by a newly planted sugarcane crop might allow the development of a high nematode population.

If soil is bare fallowed for long periods, population densities of plant parasitic nematodes are reduced and yields increase. However, the partial biological vacuum that is created results in a resurgence in populations of some plant parasitic nematodes, particularly ectoparasites such as *Tylenchorhynchus* and *Paratylenchus* (Stirling *et al.*, 2001). Long periods of bare fallow are not a sustainable option.

Rotating sugarcane with other crops and intercropping is common on the smaller farms in a number of countries, including India, Mauritius and Taiwan (Smith, 1978; Parsons, 2003). In Australia, when the sugarcane monoculture was broken with a fallow legume, yield improved by 15–25% (Garside and Bell, 2001). Since the increased yields carried through to subsequent ratoons, the sugar yield forgone by leaving land out of sugarcane for 12 months was more than recovered in the subsequent crops. Grain harvested

from the legume also adds to profitability. The choice of rotation crops depends on the target nematode species. Legumes such as groundnut and soybean reduce the populations of the lesion nematode *P. zeae* substantially, compared to continuous sugarcane (Table 18.6). Groundnut and velvet bean are effective against root knot nematodes *M. javanica* and *M. incognita*, and thus are a good choice of rotation crop for sandy soils (Stirling, 2008). In Brazil, a 2-year rotation programme with groundnut and maize proved successful in soils infested with *Meloidogyne* (de Moura, 1995). Berry *et al.* (2011) tested 27 cover crops in pot trials for their susceptibility to nematodes. All the crops hosted less *Pratylenchus* than sugarcane, and these numbers remained low even after sugarcane was planted. Cowpea, tomato and grazing vetch were good hosts for *Meloidogyne*, while oats, wheat, forage sorghum, groundnut and marigold reduced *Meloidogyne* numbers. These changes occurred within 3 months of growing these crops, and low numbers were maintained for up to 15 months of sugarcane. In addition to *Pratylenchus*, *Tylenchorhynchus* numbers were also significantly lower throughout the trial period.

Organic amendments

The addition of crop residues and animal manures to soil invariably improves plant growth, and for this reason, the practice is as old as agriculture itself. The mechanisms involved are complex and involve nutrient inputs, improvements in the cation exchange capacity of the soil, formation and stabilization of soil aggregates, improvements in water infiltration rates and water retention, and suppression of some soil-borne pathogens. Population densities of plant parasitic nematodes are usually reduced by organic amendments, and plants are better able to tolerate attack by nematodes (Stirling, 1991).

In the sugar milling process, vast quantities of crop residues are generated, in particular bagasse, which is primarily cane fibre, and filtercake (millmud), which is the sediment obtained when clarifying the juice expressed from the crushed cane (Qureshi *et al.*, 2001). There are numerous reports of the suppression of nematode populations and an increase in sugarcane yields following the addition of filtercake to the soil (Estioko *et al.*, 1988; Jonathan *et al.*, 1991; Mehta *et al.*, 1994b; Albuquerque *et al.*, 2002). Similar benefits have been reported for other locally available organic materials such as poultry manure, farmyard manure and neem (produced from *Azadirachta indica*) (Salawu, 1992; Mehta and Sundararaj, 1995). The combination of organic amendments and green manure crops has also been effective in reducing numbers of nematodes and increasing yields of sugarcane (Mehta and Sundararaj, 1997; Jonathan *et al.*, 1999). Lower rates of nematicide may be required when used in conjunction with organic amendments (Novaretti, 1992; Salawu, 1992), or greater yields may be achieved by the combination of a nematicide and an organic amendment than either on its own (Novaretti and Nelli, 1985; Cadet *et al.*, 1987).

Different types of organic matter are used to influence the nematode community in soil (see Chapter 3, this volume). In Australia, Stirling (2014) found that organic amendments with high C:N ratio, such as sawdust, sugarcane residue and grass hay, increased the suppression of plant parasitic nematodes and were more effective than nitrogenous organic matters such as feedlot manure, poultry manure, chitin and organic mill wastes. Amendment of sugarcane residue has great impact on the nematode community, increasing the population of free-living nematodes and decreasing the population of plant parasites. The variable nature of organic materials and the complex chemical and

Table 18.6. The impact of recent cropping or fallow history on nematode population densities (mean $\pm$ SE) in 49 fields on commercial farms in Australia. (From Stirling, 2008.)

Parameter	Plough-out replant <i>n</i> = 12	Non-legume fallow <i>n</i> = 12	Fallow legume <i>n</i> = 12
Plant parasitic nematodes/200 ml soil	540 $\pm$ 71	219 $\pm$ 77	303 $\pm$ 96
<i>Pratylenchus</i> /200 ml soil	305 $\pm$ 50	157 $\pm$ 75	48 $\pm$ 12
Free-living nematodes/200 ml soil	2758 $\pm$ 814	1836 $\pm$ 365	2745 $\pm$ 485

biological interactions that occur when they are added to the soil mean that responses to organic amendments are difficult to predict. In Australia, Stirling *et al.* (2003) monitored temporal changes in biological activity and suppression to plant parasitic nematodes in soils amended with sawdust, sugarcane trash, grass hay, legume hay, feedlot manure, poultry manure, chitin and millmud. Seven months after the amendments were incorporated, soils with sawdust, sugarcane trash and grass hay were more suppressive to *M. javanica* than soils amended with nitrogenous materials. Numbers of *P. zeae* in the roots of sugarcane were reduced by 60–90% in some of the amended soils. It was concluded that the quantity, quality and timing of organic inputs influenced the level of nematode control and that amended soils with a fungal dominant biology and high numbers of omnivorous nematodes were most likely to induce suppressiveness.

Resistance

Sugarcane is not attacked by a single nematode species but by a diverse community of plant parasitic nematodes. Breeding for combined resistance, even to the more important components of a community, is therefore likely to be extremely difficult (Luc and Reversat, 1985). Nevertheless, such a combination has been identified in one cultivar in Brazil, SP70-1143, as it is resistant to both *M. javanica* and *P. zeae* and tolerant of *P. brachyurus* (Dinardo-Miranda, 1994; Dinardo-Miranda *et al.*, 1995). This cultivar is widely grown on the sandy soils in Brazil where *M. javanica* is the dominant plant parasitic nematode (G.R. Machado, Brazil, 1989, personal communication). However, in a glasshouse trial in Australia, this cultivar supported a significantly large number of both types of nematodes, and was deemed to be susceptible (Bhuiyan, unpublished data). Resistance and/or tolerance to species of *Meloidogyne* and *Pratylenchus* have been identified in the cultivar collections of several countries (Suwarno, 1991; Mehta and Somasekhar, 1998; Dinardo-Miranda, 1999; Kipkorir *et al.*, 2015). Apart from *Heterodera sacchari* in Nigeria, no attempts have been made to identify resistance to species of other genera (Salawu, 1990). None the less, earlier research has suggested

that resistance to nematodes is rare in commercial sugarcane cultivars (Blair, 2005; Cadet and Spaul, 2005; Stirling *et al.*, 2011b; Santos *et al.*, 2012). This may be due to the fact that the genetic base of the modern sugarcane cultivars is narrow and could be traced back to relatively few key ancestors that were used in initial interspecific hybridizations carried out by Indonesian and Indian breeders in the early part of the 20th century (Roach, 1989). In Australia, Stirling *et al.* (2011b) showed that a wild relative of sugarcane, *Erianthus arundinaceus*, and clones derived from crosses between *Erianthus* and sugarcane had useful resistance to *M. javanica* and *P. zeae*. *Erianthus* is a source of nematode-resistance genes that could be useful in sugarcane breeding programmes. Subsequent research in Australia confirmed the findings and further revealed that *S. spontaneum*, a close relative of sugarcane, and some progenies derived from crosses between *S. spontaneum* and commercial cane possessed good nematode resistance (Croft *et al.*, 2015; Bhuiyan *et al.*, 2016b). The highest resistance to both types of nematode was observed in basic *E. arundinaceus* and *S. spontaneum*, and the levels of resistance tended to decrease with successive backcrosses between the wild species and commercial sugarcane (Fig. 18.9). None the less, a number of clones from backcross (BC) 1, BC2 and BC3 showed a high level of resistance to nematodes.

In India, Sujatha and Mehta (1998) and Kathiresan and Mehta (2002b) showed that both resistant and susceptible cultivars responded to pathogenic invasion with qualitative and quantitative changes in peroxidase and acid phosphatase activity in the roots and in the leaves.

Tolerance

While there is only a remote chance of finding cultivars that are resistant to a wide spectrum of plant-feeding nematodes, the selection of tolerant cultivars that grow well in spite of the damage caused by nematodes appears more realistic (Matsuoka, 1980). In fact, the normal selection procedures used by plant breeders tend to select such tolerant cultivars. Cultivars N12, N14 and NCo376 are tolerant of damage from nematodes (Spaul and Cadet, 2003). Similarly, CP70-321

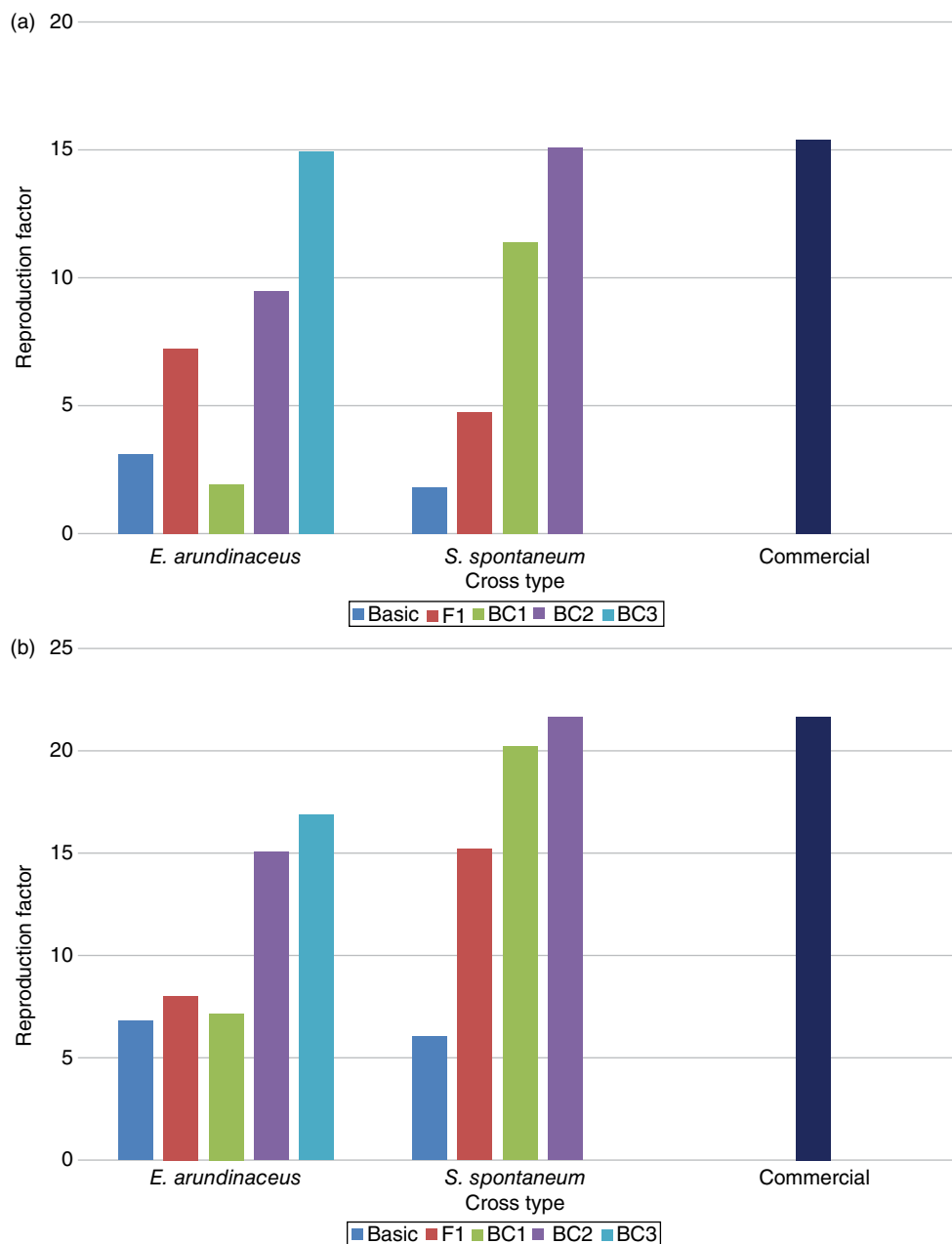


Fig. 18.9. Level of root knot (*Meloidogyne*, a) and root lesion (*Pratylenchus*, b) nematodes on various introgression cross types and commercial cultivars in Australia. BC1, BC2 and BC3 = backcross 1–3 populations, respectively.

appears tolerant to several plant parasitic nematodes, including species of *Criconeimoides*, *Paratrichodorus* and *Tylenchorhynchus* (Koenning *et al.*, 1999). In both Brazil and South Africa, it

was calculated that tolerant cultivars reduced the damage caused by nematodes from about 47% to 15% (Matsuoka, 1980; Spaul and Cadet, 2003).

Chemical control

Fumigant and non-fumigant nematicides have been used experimentally on sugarcane in many countries, particularly Australia, Brazil, Burkina Faso, India, Indonesia, Côte d'Ivoire, the Philippines, South Africa and Taiwan. In some instances, responses to treatment were good, especially on sandy soils (Spaull and Cadet, 1990). Almost all the experimental work on the chemical control of nematodes in recent times has been with non-fumigant nematicides. Chemicals investigated include aldicarb, carbofuran, oxamyl, ethoprophos, phenamiphos, terbufos and cadusafos. In many instances, treatment with these nematicides increased yield, especially on sandy soils (Bond *et al.*, 2000; Stirling and Blair, 2001; Cadet and Spaull, 2003). However, due to either their relatively high cost, their non-availability in some countries and the erratic responses that are often obtained, the commercial use of nematicides is restricted to the sandy soils of a few countries, including Australia, Brazil, Burkina Faso and South Africa. In recent years, many of the nematicides have been banned or phased out due to unacceptable non-target toxicity (Haydock *et al.*, 2013). Despite the adverse impact, nematicides are still the most effective method of controlling nematodes, often as part of integrated crop management strategies (Sikora *et al.*, 2005; Westphal, 2011) (Fig. 18.10). In Australia, no nematicides are currently registered for the control of sugarcane nematodes after the phasing out of organophosphorus nematicides. In South Africa, carbofuran, oxamyl and furaldehyde are registered for use on sugarcane. New options are available in other crops and are currently being tested in sugarcane. These include fluensulfone and fluopyram. The new nematicides have a more favourable toxicological profile than the old ones and are nematocidal instead of nematostatic. Other options being investigated in South Africa are abamectin and combinations of nematicides with insecticides.

Method of diagnosis

Sampling to determine the size and composition of the plant parasitic nematode community must be timed to take into account the dynamics of the root systems of cane. Thus, in plant cane, a representative sample of sett roots

is required. This can only be taken during the relatively short period after planting, when the cane is dependent on these roots. Samples of shoot roots can be taken at any time during the subsequent growth of the crop. In ratoon cane, the new roots attached to the developing shoots should be distinguished from the old roots of the previous crop, which may persist for several months. Soil samples to a depth of approximately 25 cm are taken close to the plant at any time during the growth of the crop. Pre-plant and mid-season threshold levels for species of *Meloidogyne* and *Pratylenchus* have been given by Stirling and Blair (2000). Diagnostic services are available in some countries, including Australia and South Africa. In South Africa and Australia, nematicide treatment is often recommended where symptoms of damage are associated with *Meloidogyne*, *Pratylenchus* and *Xiphinema*. The conventional method of diagnosis of plant parasitic nematodes relies on morphological characteristics. Such work requires skilled personnel to identify these organisms to genus and species level, and quite often requires the nematode to be at adult stage. Berry *et al.* (2007a, 2008), tested polymerase chain reaction (PCR) methods for qualitative and quantitative detection of *M. javanica*, *P. zeae* and *X. elongatum* known to be prevalent under sugarcane in South Africa. Here, discriminating characteristics such as primer specificity and PCR product melting temperature (T_m) for *M. javanica*, *P. zeae* and *X. elongatum* were investigated and tested to enumerate these nematodes in pure and mixed nematode samples extracted from soils under sugarcane. These authors concluded that there was potential to develop a single molecular test to detect multiple nematode pests of sugarcane.

Determination of crop loss

Based on estimates provided by 65 nematologists from around the world, Sasser and Freckman (1987) reported an annual loss in sugarcane production of 15.3%. This is higher than that of a number of other estimates for individual countries, i.e. Australia, 9% (G.R. Stirling, Queensland, Australia, 2003, personal communication); Peru, 3% (Carbonell, 1978); South Africa, 7.6% (Spaull and Cadet, 2003); USA, 4% (Koenning *et al.*, 1999); and Côte d'Ivoire,



Fig. 18.10. Aerial view of a nematocide trial in the KwaZulu Natal Midlands area in South Africa showing growth differences between control and treated plots.

11.0%, but similar to an estimate from Burkina Faso, 14.6% (P. Cadet, unpublished data).

The repeated application of conventional and high rates of nematicides indicates that crop loss estimates in sugarcane are much greater than those derived from treatment with a single (economic) application (Berry *et al.*, 2004) (Fig. 18.11). In addition, there are long-term consequences of the damage caused by nematodes since they not only affect the yield of each crop but also reduce the number of economic ratoons that can be harvested from a single planting (Cadet and Spaull, 2003).

Conclusion and Future Prospects

The world export price of sugar is uncertain and fluctuates constantly. In addition, production costs have increased substantially over the years. Furthermore, due to the negative perceptions associated with sugar, there has

been a huge surplus in sugar stocks. The financial return from growing sugarcane for the world market is thus much reduced. However, sugarcane remains one of the most efficient converters of sunlight, water and carbon dioxide into biomass and, unlike fossil fuels, it is a renewable resource. It is already used for a wide range of by-products (Lator, 1986; Wang, 1986; Schmitz *et al.*, 2003). Countries like Brazil switch between sugar and ethanol production, depending on which is more lucrative. In South Africa, using sugarcane to generate electricity to supply into the national grid is being investigated. Thus, despite the limitations on sugar production being profitable, the economic prospects for the use of sugarcane into the future still remain positive. It is thus paramount to maintain sustainable productivity by managing plant parasitic nematodes.

Sugarcane has historically been grown as a monoculture, and this has impacted negatively on soil health and soil degradation in



Fig. 18.11. The effect of almost complete eradication of nematodes by the repeated application of a standard rate of nematicide in a field of sandy soil in KwaZulu Natal, South Africa. The surrounding cane received only a single treatment.

many countries. Future sugarcane farming must correct these mistakes, and a more sustainable farming system should be employed. In response to the productivity plateau for the previous 20 years described in the 1990s, the Australians proposed a new farming system including minimum tillage, controlled traffic, legume break crops and the green cane trash blanket (GCTB) (Garside *et al.*, 1997). The GCTB was associated with lower multiplication of the two most damaging nematodes *P. zeae* and *M. javanica* compared to non-mulched soil (Stirling *et al.*, 2011c). The most likely cause is that C inputs from GCTB or crop residue are responsible for sustaining an active and diverse soil food web that suppresses plant parasitic nematodes (Stirling, 2014). In South Africa, sustainable farming solutions are offered through the promotion of a sustainable farm management system, SUSFARMS® (SASA, 2015). This system aims to farm sugarcane in a way that is environmentally sustainable, socially responsible and economically viable. Contained within these guidelines are practices that minimize the impact of nematodes on the crop.

The highly toxic organophosphate and carbamate nematicides used previously have been, or are being, phased out in many countries. New-generation nematicides with low impact on the environment have been, and continue to be, developed by chemical companies. The reliance on nematicides only for nematode management is declining and a more integrated approach is being investigated. Practices within this approach could include the use of endophytic and rhizospheric bacteria and fungi that are directly antagonistic to nematodes (Stirling, 2014), or nematicidal chemicals derived from such microorganisms (Hallmann and Sikora, 1996; Carneiro *et al.*, 1998). Work is also being conducted on combining biological and chemical control products within a single farming system.

Nematode management could be achieved through a low-input, integrated fertility management approach in which losses from nematodes are reduced, not completely but to levels that are both acceptable and sustainable. The key to this approach is biodiversity, which needs to be promoted at three levels: the soil microflora, the plant and the nematode community.

The objective will be to restore and sustain 'soil health' and to move away from the practice of an independent treatment for each growth impediment and regenerate a soil food web that is capable of suppressing plant parasitic nematodes (Stirling, 2014; and see Chapter 3, this volume).

1. Soil biological diversity would be enhanced if the crop residues generated from the sugar milling process were returned to the field. Interactions within such an amended soil would reduce plant parasitic nematode pathogenicity, and the nutritional benefit derived from the residues would strengthen the plant's ability to compensate for root damage. Although a better understanding of these processes is still required, encouraging results have already been obtained (Stirling *et al.*, 2003). Retaining crop residues during harvesting will also contribute to the suppressiveness of nematodes (Stirling *et al.*, 2010a).

2. At the plant level, reintroducing plant diversity in the sugarcane monoculture could be achieved with fallows and appropriate rotation or intercrops, which would also enhance biological diversity in the soil. Stirling (2008) found that breaking sugarcane monoculture with groundnut or soybean crops significantly reduced the population of *P. zeae* and *M. javanica*. In addition, Berry *et al.* (2011) showed that levels of *Pratylenchus* and *Tylenchorhynchus* were reduced significantly when growing various cover crops for 3 months, and this was sustained for 15 months of sugarcane growth. Rhodes *et al.* (2012) demonstrated that it was economically viable to take cane land out of production for a 3-month fallow; however, careful attention must be paid to the choice of green manure crop.

3. Sugarcane is usually inhabited by a wide range of different nematode genera, including both plant parasitic nematodes and free-living nematodes. Sugarcane cultivation promotes the increase of plant parasitic nematodes that cause damage to the crop. Adopting farming practices that increase organic matter in the soil will increase the free-living nematodes in the soil, thus minimizing the impact of plant parasitic nematodes.

The classical approach of selecting for nematode-resistant or -tolerant plants in plant

breeding programmes has been examined in Australia and South Africa (Cadet and Spaull, 2005; Croft *et al.*, 2015; Bhuiyan *et al.*, 2016b). Although, a large number of plant parasitic nematodes are present in sugarcane soil, root knot and lesion nematodes are the most damaging to sugarcane. Progress in introgression breeding of commercial sugarcane with wild relatives of sugarcane provides a new opportunity of developing nematode-resistant cultivars (Croft *et al.*, 2015). The classical breeding and selection for nematode resistance is rather time-consuming and expensive. Marker-assisted selection has been employed in many crops to select nematode-resistant lines in breeding programmes (Matsuo *et al.*, 2012; Shi *et al.*, 2015). In future, marker-assisted selection for identifying nematode-resistant traits in sugarcane breeding programmes should be investigated.

RNA interference (RNAi) or gene silencing is another new avenue that is being investigated. This is a biological process in which RNA molecules inhibit gene expression, typically by causing the destruction of specific mRNA molecules. This is a powerful molecular tool that can be used to regulate gene expression in which double-stranded RNA (dsRNA) can be introduced to degrade mRNA in order to prevent synthesis of the encoded protein (Jones and Fosu-Nyarko, 2014). In excess of 40 cases on successful silencing of target genes of plant parasitic nematodes from five genera by soaking in dsRNA have been reported (Lilley *et al.*, 2012). Most studies were performed on sedentary endoparasites (*Meloidogyne* spp., *Heterodera* and *Globodera* spp.). Studies on *Pratylenchus* spp. (including *P. zeae*) revealed that the effects of RNAi after feeding dsRNA with *unc87* and *pat10* genes reduced movement by >80% compared to the control population (Jones and Fosu-Nyarko, 2014). Infectivity, survival and reproduction were severely reduced in the dsRNA-fed nematodes. Although the results are encouraging for the two most important nematodes of sugarcane, *M. javanica* and *P. zeae*, the challenge is getting the nematodes to take up the exogenous dsRNA solution. Jones and Fosu-Nyarko (2014) have suggested two options: one is to add neurostimulants (resorcinol, octopamine or serotonin) to the solution containing dsRNA. This leads to an increase in pharyngeal pumping in order to increase the uptake of the external solution via the stylet.

An alternative approach is to develop transgenic crops that synthesize dsRNA homologous to a nematode gene.

Nematodes in sugarcane, as with most other crops, will never be eradicated. However, research in this area is continuing and aims to provide the sugarcane grower with a range of sustainable practices to manage this pest effectively and realize the full potential of the crop.

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19 Nematode Parasites of Tobacco*

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Tobacco (*Nicotiana tabacum*) is a high-value crop grown throughout the world for the production of cigarettes, cigars and other products, and may be the most economically important non-food crop in the world (de Araújo Filho *et al.*, 2016). Total world production in 2013 was estimated to be about 7.4 Mt, of which over 40% was produced in China (Anon., 2016). The other major tobacco-producing countries include (in order of production for 2013): Brazil, India, USA, Zimbabwe, Turkey, Indonesia, Argentina, Malawi, Tanzania and Pakistan. The sale of cured leaf and manufactured products is a major source of income for many countries, and many governments rely heavily on taxes levied on sales to consumers.

Although the word 'tobacco' usually refers to *N. tabacum*, it may also refer to *Nicotiana rustica*, which is grown for similar purposes in some parts of the world (Johnson and Reed, 1994). *N. tabacum* probably originated as a natural hybrid of *Nicotiana sylvestris* and *Nicotiana tomentosiformis* in Brazil or Central America, and has been cultivated for centuries (Ren and Timko, 2001). Tobacco cultivation was widespread in the Americas when European explorers arrived, and has since spread all over the world (Johnson and Reed, 1994). The crop is separated into 8 classes and 26 types based on plant genetics, production practices and environmental characteristics.

Cultivation techniques

Tobacco fields have traditionally been transplanted with seedlings produced in outdoor seedbeds or nurseries, but seedling production has largely switched to hydroponic systems in greenhouses or small outdoor 'float beds' (Reed, 1996). Although this change eliminates the need for pesticides and/or cultural practices to prevent early nematode parasitism of seedlings, subsistence farmers, outgrowers and others outside some of the major tobacco production areas may continue to raise tobacco transplants in outdoor seedbeds.

Tobacco is often produced in coarse textured soils with low inherent fertility in order to manage nutrient uptake by the crop more precisely. This characteristic makes the crop attractive to farmers when combined with a stably profitable demand, but warm climates and sandy soils also favour reproduction, damage by and survival of plant parasitic nematodes. Air- and fire-cured tobaccos are often grown on heavier soils, but may still suffer economic losses caused by nematodes.

Nematodes of Tobacco

Plant parasitic nematodes are found wherever tobacco is grown, but their severity depends on

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climate and soil type. A large number of tobacco-producing countries are close to, or within, the intertropical zone. The dominant nematodes there are *Meloidogyne* spp., of which the most important are *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne arenaria*. *Meloidogyne hapla* and other *Meloidogyne* spp., species of *Pratylenchus*, *Tylenchorhynchus* and *Globodera*, *Ditylenchus dipsaci* and *Aphelenchoides* may cause yield losses in certain restricted areas. Although other nematodes, such as the spiral nematodes (*Helicotylenchus*, *Rotylenchus* and *Scutellonema*), *Rotylenchulus* species, *Tetylenchus* and *Cricemoides* species occur in tobacco fields, they are not normally associated with losses. Some species of *Xiphinema*, *Longidorus*, *Trichodorus* and *Paratrichodorus* are reported to transmit viruses to tobacco. More recently, *Heterodera glycines* and *Radopholus similis* have both been reported to parasitize tobacco in China (Han *et al.*, 2009; Cheng *et al.*, 2012; Shi and Zheng, 2013).

Meloidogyne

Root knot nematodes (*Meloidogyne* spp.) likely damaged tobacco since the crop was introduced to the south-eastern USA, but early reports on root knot damage and control recommendations may have been published first from Florida and Georgia (Boyd, 1925; Shepherd and Barker, 1990). Root knot nematodes were also recognized as serious pests in southern Africa in the late 1920s, and have long been considered important pests in most of the tobacco-growing countries of the tropical and subtropical zone.

A large number of *Meloidogyne* species reproduce on tobacco, but not all are economically important. *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* have been associated most frequently with tobacco, with *M. incognita*, *M. javanica* and race 2 of *M. arenaria* considered the most important, due to their widespread distribution, relative reproductive capacity and damage potential (Barker and Lucas, 1984; Johnson, 1998). Juveniles of *M. javanica* can locate and invade tobacco roots more quickly and in larger numbers than *M. arenaria*, which invades roots at a more rapid rate than does *M. incognita* (Johnson, 1998). Differences in fecundity were not found among the three species. However, differences in typical gall size, syncytial shape and

structure and extent of associated root necrosis among the three species may indicate additional differences in the physiological effects of *M. arenaria*, *M. javanica* and *M. incognita* (Johnson, 1998).

M. incognita and *M. javanica* are the most widely distributed of the important root knot species (Table 19.1). Their relative importance is largely dependent on the climate, since *M. javanica* has a greater tolerance to drought and high temperature than *M. incognita* (Shepherd and Barker, 1990). *M. arenaria* and *M. hapla* are the next most widely distributed root knot species, with *M. hapla* largely confined to cooler temperate conditions. The relative distribution of root knot species appears to be changing in some areas, perhaps due to the long-term planting of cultivars resistant to races 1 and 3 of *M. incognita* (Eisenback and Johnson, 2012). *M. arenaria*, *M. javanica* and race 2 of *M. incognita* occur in Cuba (Fernández and Ortega, 1998). Although *M. javanica* was previously identified in 50% of cases in Brazil, *M. incognita* in 20% and both together in 25% of samples, later surveys found *M. incognita*, *M. javanica*, *Meloidogyne enterolobii*, *M. arenaria* and *Meloidogyne inornata* in 46%, 41%, 26%, 5% and 3% of samples, respectively (Johnson *et al.*, 2005; de Araújo Filho *et al.*, 2016). *Meloidogyne* spp. (*M. incognita* races 1 and 2 and *M. javanica*) are nearly ubiquitous in tobacco fields in Colombia (Barriga-Olivares and Aranda-Ramirez, 2000).

M. javanica is the dominant problem in southern Africa, but *M. incognita* is also common and important in Zimbabwe (Table 19.1; Shepherd and Barker, 1990). Root knot can be a serious problem on tobacco in areas of Mozambique (van den Oever *et al.*, 1998). *M. incognita* and *M. javanica* have caused heavy losses to tobacco in Nigeria (Khan, 1990).

Meloidogyne spp. are common in Italian tobacco fields but are only a problem in sandier soils in northern Italy (Table 19.1; Johnson *et al.*, 2005). *M. arenaria* predominated in the La Vera region of Spain after widespread planting of flue-cured tobacco cultivar K 326, resistant to races 1 and 3 of *M. incognita* (Navas *et al.*, 2001; Flores-Romero *et al.*, 2006).

Nematodes were first reported on tobacco in Sichuan, China, in 1939, were detected in Shandong, Henan and Anhui provinces through the 1980s, and since in Yunnan, Guizhou, Guangxi, Fujian, Hubei, Hunan and Shanxi provinces, with significant losses reported from Yunnan, Sichuan,

Table 19.1. Importance of *Meloidogyne* species and *Pratylenchus* in some tobacco-growing countries. (From 2015 CORESTA Agro-Chemical Advisory Committee (ACAC) survey of tobacco industry contacts.)

Country	Area affected (%)	Species severity (1 = low; 5 = high)					
		<i>Meloidogyne</i> spp.					<i>Pratylenchus</i> spp.
		<i>Meloidogyne arenaria</i>	<i>Meloidogyne incognita</i>	<i>Meloidogyne javanica</i>	<i>Meloidogyne hapla</i>	<i>Meloidogyne exigua</i>	
Brazil	<1	1	1	1			
Bulgaria	0						
Canada	100						Present
Yunnan, China	<1	4	2	1			
Germany	<1						Present
Guatemala	60		3			2	3
Indonesia	30		3	3			
Umbria, Italy	30	1	4				
Veneto, Italy	80	1	4				
Macedonia	<1	1	2	1	1		
Malawi, large-scale farms	100			1			
Malawi, small-scale farms	80			2			
Serbia	0						
Tanzania	5						
Thailand	0						
Turkey, Aegean region	1			1			
Uganda	10			2			
USA (flue-cured in NC, SC, GA)	74	3	4	1			
Zambia, flue-cured	70	5	5	5			
Zambia, burley	100	5	5	5			
Zimbabwe	100		3	3			

Hubei, Henan and Shandong. Nematodes, primarily *Meloidogyne* spp., damaged over 10,000 ha between 2011 and 2015, annually reducing yields by nearly 5800 t and economic value by more than 68 million RMB (Chen, China, 2016, personal communication). *M. incognita* is the dominant species on tobacco in Shanxi, Henan, Guizhou and Sichuan (Chen *et al.*, 1991; Jiang and Xing, 1992; Huang *et al.*, 2009; Wang *et al.*, 2010; Jiao *et al.*, 2014; Mo *et al.*, 2016), while *M. arenaria* is the major root knot problem in Yunnan and is important in other provinces, such as Hubei. *M. javanica*, *M. incognita* (*acrita*) and *M. hapla* have also been reported as damaging tobacco in China (Li *et al.*, 2012; Dong *et al.*, 2014; Anon., 2015).

M. incognita, *M. arenaria*, *M. javanica* and *Meloidogyne thamesi* are important problems on tobacco in India. *M. incognita* may predominate over *M. javanica* in the Philippines, and occurs in Sri Lanka (Johnson *et al.*, 2005). Research on resistance to *Meloidogyne* spp. in tobacco cultivars has also been reported from Iran (Honarnejad and Shoaee-Deylami, 1997). *Meloidogyne microcephala*, *Meloidogyne mayaguensis*, *Meloidogyne cruciani*, *M. enterolobii*, *Meloidogyne ethiopica*, *Meloidogyne paranaensis*, *Meloidogyne petuniae*, *Meloidogyne platani*, *M. thamesi*, *Meloidogyne brasiliensis* and *Meloidogyne* sp. are also reported to reproduce on tobacco, but their importance is very restricted (Johnson *et al.*, 2005).

Symptoms of damage

The characteristic symptoms of root knot nematode attack are the root galls formed as a reaction to the invasion and feeding by the nematode (Fig. 19.1a). These can range from small individual galls to severe distortion and restriction of root development. The size and

magnitude of the galls can be a guide to the species involved. Galls induced by *M. hapla* are usually small and affect only a limited portion of the root system. *M. arenaria* causes bead-like galls, which may involve a large proportion of the root system. Conversely, *M. incognita* and *M. javanica* cause large galls, which may affect 90% or more of the root, with the latter usually causing the more extensive gall formation. Root decay often develops in roots galled by *M. javanica*, *M. incognita* and *M. arenaria* (Fig. 19.1b), whereas decay is usually less severe in roots infected by *M. hapla*.

The above-ground symptoms of a severe attack are stunted growth (Fig. 19.2), often associated with premature wilting, typically in the afternoon on hot days (Fig. 19.3). Even low densities of *M. incognita* delay and reduce tobacco flowering (Preisser *et al.*, 2007). These symptoms are often seen in a patchy distribution in the field, unless the infestation is uniformly severe. There may also be signs of nitrogen and potassium deficiency and scorching of the leaf tips and margins. Weeds, which are usually out-competed by healthy tobacco plants, are able to grow successfully and compete for soil moisture and nutrients. Sucker development is also much suppressed on plants heavily parasitized by nematodes.

Survival and dissemination

All of the root knot nematodes that attack tobacco have a wide host range and can survive between tobacco crops on many weeds and other crops, especially if tobacco is grown frequently in the same field. The nematodes also spread in soil, remaining on field equipment after cultural operations have been performed,

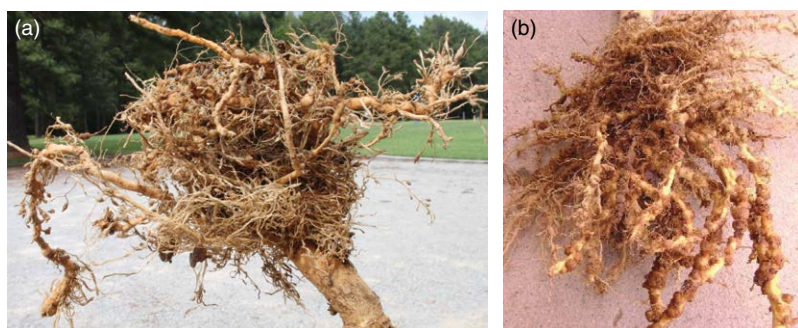


Fig. 19.1. Root symptoms of parasitism by *Meloidogyne* spp. on tobacco: (a) typical galling; (b) galling and associated necrosis. (Photographs courtesy of C. Johnson (a) and C. Chinheya (b).)



Fig. 19.2. Typical irregular pattern of stunting in flue-cured tobacco parasitized by root knot nematodes. (Photograph courtesy of C. Johnson.)



Fig. 19.3. Chlorosis and scorching of leaf margins and tips due to root knot parasitism. (Photograph courtesy of C. Johnson.)

and as eggs and juveniles in irrigation water applied to fields or seedbeds. Although bore-hole (well) or mains (publicly supplied) water should be nematode free, surface water from streams or lakes can become contaminated when infested soil is washed into the water source during heavy rain. Root knot nematodes can also be spread by using improperly prepared compost or dung from animals fed on infected root crops (Shepherd and Barker, 1990).

Disease complexes

Interactions with other microorganisms play an extremely important role in the epidemiology and management of *Meloidogyne* spp. on tobacco. Root knot nematode parasitism can exert local and systemic effects on tobacco, changing the structure and function of the root system as well as the physiology of the entire plant, encouraging infection by other pathogens (Johnson, 1998). *M. incognita* does not compete with the parasitic plant *Orobanche ramosa* for infection sites on tobacco, so damage from concomitant attack from the two pests is additive (Johnson, 1998). Parasitism by *Meloidogyne* spp., however, increases plant mortality caused by soil-borne fungi, such as *Phytophthora nicotianae*, causal agent of black shank, one of the most common and damaging diseases of tobacco worldwide (Shepherd and Barker, 1990). Although resistance to root knot can limit black shank development, resistance to black shank does not prevent root knot invasion and subsequent damage (Johnson, 1998). Control of the black shank-root knot disease complex depends on crop rotation and the use of cultivars resistant to both black shank and root knot and/or the application of soil pesticides registered for control of both organisms. Increased incidences of Fusarium wilt (*Fusarium oxysporum* var. *nicotianae*) and bacterial wilt (*Ralstonia solanacearum*), often referred to as Granville wilt, are also associated with root knot nematode infection (Shepherd and Barker, 1990). Control of these diseases, where they occur, can be extremely difficult, requiring the use of extended crop rotation intervals and high rates of soil fumigants with highly resistant cultivars.

Parasitism by *Meloidogyne* spp. can also enable soil-borne microorganisms that are not normally pathogens of the crop to infect and damage

tobacco roots. *Rhizoctonia solani* and species of *Curvularia*, *Botrytis*, *Aspergillus*, *Penicillium* and *Trichoderma* caused root necrosis severe enough to result in above-ground symptoms, but only when inoculation was preceded by infection by *M. incognita*. Although increased stem damage by the sore shin fungus *R. solani* has been reported when combined with root knot infection, others indicated no consistent effect of root invasion by *M. javanica* on stem damage by *R. solani* (Shepherd and Barker, 1990).

Root knot parasitism can also exacerbate foliar disease problems. Infection by *M. incognita* predisposes tobacco plants to brown spot caused by *Alternaria alternata*, and the root knot-Fusarium disease complex worsens this effect (Barker and Lucas, 1984; Shepherd and Barker, 1990). Infection by *Meloidogyne* spp. may also increase ozone injury (Johnson, 1998). The incidence of Tobacco mosaic virus (TMV) may increase in plant populations that are parasitized by root knot nematodes (Patel and Patel, 1994b). Combined infection by these two pathogens reduces plant growth and yield more than infection by either pathogen alone and alters tobacco leaf chemistry, but these interactions do not appear to be synergistic (Kartono, 1980; Patel and Patel, 1994a,b, 1995). Higher densities of *M. javanica* have been noted in tobacco plants infected with TMV (Goswami and Raychaudhuri, 1973). Tobacco cultivars resistant to races 1 and 3 of *M. incognita* react with a systemic necrosis to infection by the MN strain of Potato virus Y (PVY), a possible pleiotropic effect of the root knot resistance gene (Johnson, 1998).

Root knot nematodes occur as populations of a single species or as communities of several species, and must often compete with each other and with other plant parasitic nematodes (Johnson, 1998). Antagonistic interactions within mixed populations of root knot nematode species may result from competition for feeding sites, further complicated by differences among species in adaptation to environmental conditions (Johnson and Nusbaum, 1970; Hirunsalee *et al.*, 1995b; Ng'ambi *et al.*, 1995). Greenhouse studies indicate that *M. arenaria* outcompetes races 1 and 3 of *M. incognita* in parasitizing tobacco roots (Ng'ambi *et al.*, 1995; Johnson, 1998). Although race 1 of *M. arenaria* can damage tobacco, race 2 is the more damaging of the two host races (Hirunsalee *et al.*, 1995a,b). Root

knot and lesion nematodes may also compete with each other for penetration sites on tobacco roots (Olthoff *et al.*, 1973). Reproduction of *M. hapla* can be inhibited by concomitant infection by *M. incognita* or *Pratylenchus brachyurus*. Density increases of both *M. incognita* and *P. brachyurus* may also be suppressed when these two species occur together. Similar interactions have been observed among *M. incognita*, *M. javanica* and the reniform nematode *Rotylenchulus reniformis* (Patel and Patel, 1991).

Economic importance

Root knot nematodes are common pests of economic importance in tobacco culture, particularly where temperature and soil type favour them (Shepherd and Barker, 1990). They are of limited importance in colder areas, such as Canada, where mainly *M. hapla* occurs, or France, where *M. incognita* and *M. arenaria* have been found on tobacco.

Extensive research has been conducted to estimate crop losses in tobacco that result from parasitism by *Meloidogyne* species (Johnson, 1998). The relationship between nematode reproduction and yield loss in tobacco is greatest for *M. javanica*, followed by *M. arenaria*, *M. incognita* and *M. hapla*. Losses are generally greatest for tobacco planted in sandy versus clay soils, and when environmental conditions stress the crop (Johnson, 1998). Resistant cultivars suppress nematode reproduction and increase yield, but may still suffer yield loss when initial densities are high, perhaps due to the hypersensitive reaction of resistant roots to attempts by the nematodes to establish feeding sites (Sosa-Moss *et al.*, 1983).

Daulton (1963) stated that field fumigation could increase cured leaf yield in Zimbabwe by 55–1800 kg/ha by controlling *M. javanica*. Currently, yields on small-scale tobacco farms in Zimbabwe (~42% of the crop) average 68% less than yields from commercial-scale farms, largely due to poor nematode control. Fumigation suppressed galling by 92% and 30% on a susceptible and resistant cultivar, respectively, while a contact nematicide suppressed galling and increased yield by 10–11% on the susceptible cultivar, with less impact on the resistant line (C. Chinheya, Zimbabwe Tobacco Research Board, 2016, personal communication).

Average tobacco losses from root knot nematodes in the USA have remained low over the past 30 years due to improved nematode management. Annual loss estimates for North Carolina have averaged around 1% or lower since 1970. Root knot nematodes have been estimated to cause yield losses of 50–60% in some parts of Turkey (Shepherd and Barker, 1990). In Iraq, more than 40% of the tobacco was reported to be infested, with infestation levels up to 100% in some fields, while in India a 25% loss was reported from infestations originating from the field and a 50% loss when originating from a seedbed.

Threshold damage levels provide useful guides for managing *M. incognita* with minimal use of pesticides, but nematicide use is commonly recommended whenever *M. arenaria*, *M. javanica* or important root disease complexes are detected (Ye, 2011; Peterson, 2015). A combination of root examination and soil assay results is now recommended in some areas of the USA to assist farmers in determining which measures to employ for nematode control in the current crop (Johnson, 2017).

Pratylenchus

Pratylenchus species are migratory endoparasitic 'lesion nematodes' that cause brown root rot of tobacco. Lesion nematodes are less important in the tropical and subtropical regions than the root knot nematodes, but are responsible for significant yield losses in other tobacco-growing areas, such as Canada (see Table 19.1; Anon., 2015). Eleven species (*Pratylenchus alleni*, *P. brachyurus*, *Pratylenchus crenatus*, *Pratylenchus hexincisus*, *Pratylenchus neglectus* (*minyus*), *Pratylenchus penetrans*, *Pratylenchus pratensis*, *Pratylenchus scribneri*, *Pratylenchus thornei*, *Pratylenchus vulnus* and *Pratylenchus zeae*) are reported from tobacco soils from around the world, but the damage potential of *P. alleni*, *P. brachyurus*, *P. scribneri* and *P. zeae* is unclear (Gao *et al.*, 1994; Johnson, 1998). Populations of *P. penetrans* from tobacco fields can develop insensitivity to nicotine, perhaps explaining why this species is the dominant lesion nematode in Canadian tobacco fields (Yu and Potter, 2008).

Above-ground symptoms of *Pratylenchus* attack are very similar to those caused by other tobacco nematodes (Fig. 19.4a). Macroscopic

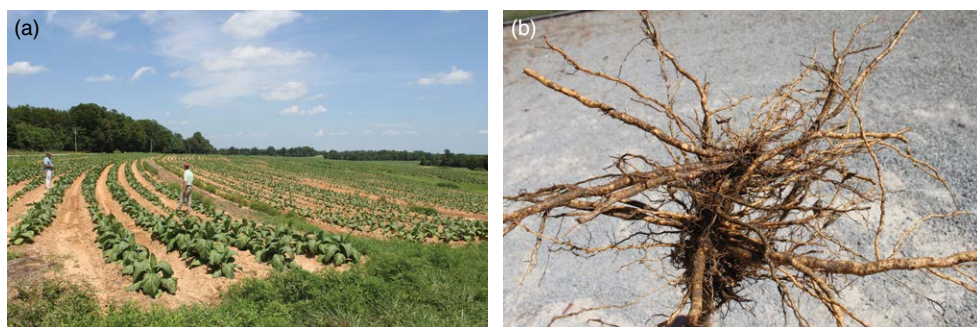


Fig. 19.4. Symptoms of *Pratylenchus* parasitism of flue-cured tobacco in Virginia: (a) irregular pattern of stunting; (b) numerous discrete necrotic lesions on lateral roots of a parasitized plant. (Photographs courtesy of C. Johnson.)

root symptoms are also very similar to those of black root rot caused by *Thielaviopsis basicola* (syn. *Chalara elegans*) (Fig. 19.4b). Root lesions caused by *Pratylenchus* spp. first appear as discrete water-soaked areas that attain a yellow colour which darkens to brown over time. Lesions may coalesce to encircle infected roots, causing the cortex to slip off and leave only the vascular cylinder remaining.

The migratory endoparasitic lifestyle of *Pratylenchus* spp. may cause it to interact differently with other soil-borne pathogens of tobacco compared with the sedentary endoparasitic *Meloidogyne* spp. Simultaneous inoculation of black shank-susceptible tobacco with *P. brachyurus* and *P. nicotianae* increased black shank development and severity, but inoculation with *P. brachyurus* or *P. penetrans* prior to *P. nicotianae* reduced black shank symptom severity and disease incidence (Inagaki and Powell, 1969; McIntyre and Miller, 1978). Lesion nematode infection did not increase black shank severity on black shank-resistant cultivars. Root wounding due to nematode penetration may have facilitated fungal infection of simultaneously inoculated susceptible cultivars, but prior infection by *P. penetrans* appeared to induce a general, systemic and sustained host response to attack. *P. hexincisus* was associated with the development of 'black root rot' (*T. basicola*) in the black turf soils of South Africa when they were wet at planting time (Shepherd and Barker, 1990). Concomitant infection of lesion and root knot nematodes can mutually suppress reproduction, but the specific resistance characteristics of the cultivars involved can change the characteristics of these interactions significantly (Johnson, 1998). *P. penetrans* can

also be suppressed by concomitant infection with *Tylenchorhynchus claytoni* and *Globodera tabacum tabacum* (Johnson, 1998).

Although lesion nematodes are rarely considered major tobacco pests (see Table 19.1; Johnson *et al.*, 2005), they can cause significant yield losses when they occur in large densities (500–1000/kg soil) (C. Saude, Ontario, 2016, personal communication) and under suitable environmental conditions (Olthoff *et al.*, 1973). Losses in gross economic returns were estimated at 11 and 27.5% when initial population densities were 6000 and 18,000/kg soil, respectively. Almost all of the flue-cured tobacco acreage in Ontario, Canada, may suffer from brown root rot (H. Papenfus, Agrochemical Advisory Committee of Cooperation Centre for Scientific Research Relative to Tobacco, 2016, personal communication). *Pratylenchus* spp. often have a wide host range, and because they can overwinter in plant roots and withstand desiccation, they can remain viable between tobacco crops.

Globodera

Various tobacco types produced around the world are attacked by members of a species complex of round cyst nematodes that may have originated in the Andes Mountains of South America (Grenier *et al.*, 2010). Problems with these nematodes have been reported from over 12 tobacco-producing countries (Johnson *et al.*, 2005; Bélair and Miller, 2006; Alenda *et al.*, 2014). Species within this tobacco cyst nematode (TCN) complex vary in host range, but none

infect plants other than tobacco and certain members of the Solanaceae, and can be identified using a variety of techniques (Skantar *et al.*, 2007; Nakhla, *et al.*, 2010; Handoo *et al.*, 2012). TCNs have been reported to occur in Morocco, but not from southern Africa (Johnson *et al.*, 2005). Limited reproduction by *Globodera pallida* and *Globodera rostochiensis* on tobacco has been reported but never confirmed (Meredith, 1976; Parrott and Miller, 1977). The taxonomy within the complex has been clarified most recently by evaluating the sequence variability of three genes coding for cell wall degrading enzymes important in TCN juveniles penetrating and migrating through host roots early in the parasitic process, and two genes involved in syncytial (TCN feeding site) formation (Alenda *et al.*, 2013). *G. t. tabacum* has been an important pest of shade-grown tobacco in Connecticut, USA, since 1951 and is the most frequently reported TCN subspecies around the world (Chaves, 1987; Johnson *et al.*, 2005; Alenda *et al.*, 2013). *Globodera tabacum solanacearum* attacks flue-cured tobacco in Virginia, some counties in North Carolina, USA, and may be present in Mexico, but has not been

found outside the Americas (Johnson *et al.*, 2005; Alenda *et al.*, 2013). *Globodera tabacum virginiae* also occurs in Virginia and North Carolina and was recently detected in France, but does not attack flue-cured cultivars and reproduces slowly on burley tobacco (Shepherd and Barker, 1990; Bardou-Valette *et al.*, 2016). Crop production practices, soil temperature and antagonistic and/or competing nematode genera are important factors influencing the spread of this nematode (Rideout *et al.*, 2000a; Alenda *et al.*, 2014).

Above-ground symptoms of TCN parasitism involve irregularly distributed crop stunting, similar to that associated with severe root knot and lesion nematode infestations, but do not include premature foliar chlorosis often seen from root knot parasitism (Fig. 19.3 and Fig. 19.5a). However, TCN-infected plants have small root systems with cysts attached to them, while significant root rot is not observed unless a root disease complex is operating. TCN cysts are barely visible (0.5 mm) to the naked eye; each may contain several hundred eggs. Mature females are usually pearly white (Fig. 19.5b), but after the female dies, her cuticle oxidizes and changes



Fig. 19.5. Symptoms of parasitism of flue-cured tobacco by *Globodera tabacum solanacearum* in Virginia, USA: (a) irregular pattern of stunting; (b) mature white female nematodes on roots of a tobacco transplant; (c) mature cysts of *G.t. solanacearum* on tobacco roots. (Photographs courtesy of C. Johnson.)

colour to a reddish brown (Fig. 19.5c). The eggs within these cysts can survive for 11 years or more until stimulated to hatch by temperature and host root exudates. The protection afforded by the cysts to the eggs within them is thought to be responsible for the long viability of the eggs, and also explains why eggs/J² within cysts are so difficult to kill with nematicides (Johnson, 1998).

The TCN complex is often associated with increased damage from bacterial wilt, black shank and Fusarium wilt (Elmer *et al.*, 1980; Johnson, 1998) (Fig. 19.6). TCN increases disease in these interactions via a localized, rather than a systemic, effect. *G. t. tabacum* increases root infection by *F. oxysporum* to a greater extent than *M. hapla*, although on wilt-susceptible but not resistant tobacco (LaMondia, 1995b). TCNs may also suppress the reproduction of mycorrhizal fungi, which are important in the normal development of tobacco (Johnson, 1998). TCNs interact with concomitant infection with other plant parasitic nematodes, such as *Pratylenchus* spp. (Barker and Lucas, 1984). Although high densities of *P. penetrans* can slow TCN population increase, moderate to high TCN densities can suppress the reproduction of *P. penetrans* until the latter become undetectable.

Yield losses of tobacco infected with *G. t. tabacum* can be very high. Virginia farmers have recorded complete crop failures, but losses can average 15% (Johnson, 1998). High TCN densities early in the growing season can reduce flue-cured tobacco yield by 25–50%, although tobacco may escape significant losses from moderate populations, especially under favourable growing conditions (Johnson, 1992). Although the relationships between TCN and flue-cured tobacco yield may vary considerably across years and cultivars, consistently negative correlations have been observed between fresh leaf weight and TCN density in soil 6 weeks after transplanting (Wang *et al.*, 1999). Although initial densities of *G. t. tabacum* below 100 juveniles/cm³ soil may reduce shade tobacco leaf yield by less than 5%, densities between 500 and 1000 juveniles/cm³ can reduce yield by 45% (Johnson, 1998). Initial TCN densities below 50 juveniles/cm³ soil can reduce the shoot weight of shade and broad-leaf tobacco by 39 and 14%, respectively (LaMondia, 2002a). Densities above 600 TCN juveniles/cm³ reduced the shoot weight of shade and broadleaf tobacco by 60 and 40%, respectively.



Fig. 19.6. Plant mortality in flue-cured tobacco caused by a *Meloidogyne* spp.–*Phytophthora nicotianae* (root knot–black shank) nematode–disease complex. (Photograph courtesy of C. Johnson.)

Heterodera

Populations of a lemon-shaped cyst nematode were reported to be parasitizing multiple tobacco fields in Shandong, China, and in Henan Province (Cheng *et al.*, 2012; Shi and Zheng, 2013). The population in Shandong was identified as *H. glycines* (soybean cyst nematode – SCN), based on nematode morphology and molecular diagnostics. The tobacco isolate of SCN from Shandong province was reported to undergo four generations per year on tobacco (Zhao *et al.*, 2013). Excessive soil moisture during plant growth was found to reduce the number of successful nematode generations, which was also found to correlate inversely with the nicotine content in roots and leaves (Dong *et al.*, 2015).

The Henan population showed 99% sequence similarity to *H. glycines* strain Hg1-Ark1, while a duplex PCR obtained a 477-bp fragment specific for *H. glycines*. The population reproduced on tobacco, but not soybean in a greenhouse pot experiment, and was proposed to be a new SCN pathotype (Shi and Zheng, 2013). SCN populations collected from tobacco and soybean in Shandong province were morphologically similar, but populations collected from soybean could infect and develop into females on soybean cv. Hedou 12, while populations collected from tobacco and rehmanna could not (Wang *et al.*, 2014, 2015). An ‘SCN-tobacco’ isolate multiplied more on soybean cultivar Lee than on the other host differentials, and on cultivar Peking more than on cultivar Pickett and PI90763, and least on PI88788. Female densities of the tobacco SCN isolate were greater on US flue-cured tobacco cultivar K 326 (31) and intermediate on US cultivar NC 95 compared to NC 89 (15) (Liu *et al.*, 2016).

Rotylenchulus reniformis

R. reniformis limits seed germination, nutrient uptake and growth of tobacco seedlings in India, as well as yield and value (Johnson, 1998). This nematode has also been reported on tobacco in Trinidad and North Carolina, but research in North Carolina indicated only moderately increased yield and value arising from nematicide use and suggested that tobacco might not be a

good host for *R. reniformis* (Melton and Powell, 1991). Both *M. incognita* and *M. javanica* damage tobacco more than *R. reniformis*, and can also suppress reproduction of the reniform nematode (Johnson, 1998).

Ditylenchus

D. dipsaci, the stem and bulb nematode, occurs in many countries, but yield loss in tobacco has only been reported from the Netherlands, France, Germany, Switzerland and Serbia (Shepherd and Barker, 1990; Johnson, 1998). Isolates of *D. dipsaci* extracted from tobacco may not parasitize other crops such as wheat (*Triticum aestivum*), maize (*Zea mays*) or sugarbeet (*Beta vulgaris* subsp. *vulgaris*) (Johnson, 1998). *D. destructor* has also been reported to reproduce to a limited extent on some tobacco genotypes (Johnson, 1998). Invasion of the lower parts of the stem by the nematode causes ‘stem break’, which is very rarely found in subtropical or tropical countries. The nematode can remain dormant in a cryptobiotic stage for many years and withstand freezing. ‘Stem break’ is usually associated with cool, damp weather and heavy soils, and is only of localized importance, but has been reported to cause losses of up to 54% in parts of north-east France (Shepherd and Barker, 1990).

Other nematodes

Various other nematodes have been associated with tobacco in isolated reports or from very restricted production areas. *Aphelenchoides* species have been reported on tobacco in France, Germany, China, Pakistan, Brazil and Chile (Johnson *et al.*, 2005). In France, *Aphelenchoides ritzemabosi* has been described as the cause of ‘checkered leaf disease’ in a localized area near the Atlantic end of the Pyrenees (Shepherd and Barker, 1990). The polygonal leaf blotches bounded by the veins caused by *A. ritzemabosi* are similar to those it causes in chrysanthemum. Various species of *Tylenchorhynchus* are reported from tobacco in New Zealand, Canada, the USA and India (Shepherd and Barker, 1990; Patel and Patel, 1992, 1999). In India, *Tylenchorhynchus vulgaris* has been reported to reduce plant

growth and nicotine content, and to interact with *Pythium aphanidermatum* to reduce seedling vigour and stand in bidi tobacco plant beds (Patel and Patel, 1993, 1998). Stunt nematodes have been reported to increase the incidence of Fusarium wilt but not Granville wilt, and may not damage tobacco directly (Shepherd and Barker, 1990). The spiral nematode is frequently reported from tobacco soil, and *Scutellonema brachyurus* has been reported to reduce growth, but it is considered a very minor pest. A range of ectoparasitic nematodes were detected in the rhizosphere of tobacco in a 2010–2011 survey in Yunnan, China, including two species of *Aphelenchus* and of *Helicotylenchus*, *Filenchus heterocephalus* and *Tylenchorhynchus spinicaudatus* (Liu *et al.*, 2012). Greenhouse pot research found reproduction ratios on tobacco ranged from 1.1 to 1.8 for ten imported populations of *R. similis* (Han *et al.*, 2009).

Various nematode species may damage tobacco by vectoring plant viruses (Johnson, 1998). *Paratrichodorus* and *Trichodorus* species vector the 'tobacco rattle' virus in parts of the Netherlands and Germany, and *Xiphinema* and *Longidorus* species are widespread and are also virus vectors. *Paratrichodorus lobatus* is also reported to cause stunting of tobacco in Australia. *Xiphinema americanum* is a relatively efficient vector of the Tobacco ringspot virus, which is reported in many countries and has localized importance. *Longidorus elongatus* is also reported to damage tobacco in Canada (Shepherd and Barker, 1990).

Management measures

Nematode management measures for tobacco vary widely around the world. Losses to nematode damage are considered slight in some tobacco-growing regions, and little attention is paid to nematode management. Such regions are often those where tobacco is grown under cool conditions, on heavier soil types, or where the root knot nematode is not widely distributed. However, there are still many countries where nematodes do cause economic losses and little attention is paid to nematode control, especially where itinerant farmers possess insufficient resources to purchase inputs for effective control measures. In other areas, cultural practices,

such as crop rotation and host resistance, are sufficient to limit crop losses to acceptable levels. However, in production regions such as the USA and parts of China and Central and southern Africa, where tobacco is an extremely important cash crop and where nematodes, especially root knot, are widely distributed, nematode management constitutes a critical role in tobacco production. The basic strategy for nematode control for tobacco, in general, is to reduce the initial soil densities and/or in transplants and to reduce the subsequent rate of nematode increase.

Cultural control

Cultural practices, such as the early destruction of tobacco roots after harvest, early and deep ploughing of tobacco fields into high, wide planting ridges before transplanting, early planting and the use of appropriate cover and rotation crops provide the foundation for consistently effective nematode management in tobacco (Shepherd and Barker, 1990; Johnson, 1998). Early destruction of tobacco roots limits nematode reproduction after harvest has been completed, reducing soil densities before the following tobacco crops. Early and deep ploughing of fields exposes nematodes to adverse temperature and moisture conditions, particularly when pre-transplant cultivation builds elevated beds or ridges into which the crop will be transplanted (Johnson, 1998). Early transplanting may enable tobacco seedlings to begin establishing a functional root system when temperatures in the soil are less favourable for nematode hatching and migration, but may expose the crop to threats from other pathogens and pests. Farmers unable to purchase expensive nematicides or too dependent on economic returns from tobacco to plant low-value rotation crops can use these methods to reduce, but not eliminate, their losses to nematode parasitism. Cultural nematode management practices also enable farmers who use crop rotation and nematicides to maximize the nematode control benefits from these practices.

Tobacco is rotated with other crops on an estimated 60% of the fields where the crop is produced around the world, reducing pre-plant soil densities of plant parasitic nematodes (Papenfus and Jack, 2013). Unfortunately, continuous tobacco production remains the norm in some important production regions, especially

where small-scale farming is common (Papenfus and Jack, 2013). The effectiveness of crop rotation varies for different nematode species, but the most common rotation crops are maize, small grains, pasture grasses and rice (Papenfus and Jack, 2013). Nematode management benefits from crop rotation must be balanced with economic and environmental considerations, but rotation intervals typically range from a single commercial crop alternating with tobacco to periods of 3–4 years, often including a pasture grass (Papenfus and Jack, 2013). Bare fallow reduces nematodes but provides no economic return and promotes soil erosion (Johnson *et al.*, 2005). Weedy fallows can allow nematodes to increase on alternative hosts, particularly *Meloidogyne* and *Pratylenchus* spp. (Johnson *et al.*, 2005; Kokalis-Burelle and Rosskopf, 2012).

Meloidogyne and *Pratylenchus* spp. have wide host ranges, but with significant differences in host range among the species within each genus (Johnson, 1998). *Globodera* species do not have wide host ranges, but can survive for extremely long periods between host crops. The choice of rotation crops is also more difficult when mixtures of root knot nematode species are present. Small grains and forage grasses, such as fescue (*Festuca pratensis*), are recommended commonly to reduce root knot and cyst nematodes in tobacco fields, although the choice of crops, and even cultivars, to rotate with tobacco should depend on the most important nematode species and races present and the economic circumstances of the grower (van Biljon, 2010; Peterson, 2015; Mila, 2016; Johnson, 2017). Managing nematode-susceptible weeds in rotation crops can also be important (Clayton *et al.*, 1944). Pasture grasses protect the soil from erosion better than row crops, and if sown densely enough, will smother weeds that might be nematode hosts. In southern and Central Africa, the Ermelo and Umgeni strains of weeping lovegrass (*Eragrostis curvula*), Katambora Rhodes grass (*Chloris gayana*) and Sabi Panic grass (*Panicum maximum*) are recommended (Shepherd and Barker, 1990; Chinheya, unpublished data). The sunn hems (*Crotalaria* spp.) can be planted in Zimbabwe as a relay crop to reduce root knot galling on tobacco (Shepherd and Barker, 1990). The toxins produced by some *Crotalaria* spp. are toxic to livestock, and the plants may persist as weeds in subsequent crops (Johnson, 1998).

Also, the increased nitrogen status of the soil after a legume is not always desirable for flue-cured tobacco.

Maize is often grown in tobacco rotations, and resistant cultivars can lower densities of *M. javanica* to levels easily controlled by nematocides if grown for 2 years or more (Shepherd and Barker, 1990). However, maize is not generally recommended for root knot control in the USA, due to the varying degrees of susceptibility among cultivars to all of the common *Meloidogyne* spp., except *M. hapla* (Peterson, 2015). Grain sorghum suppressed South Carolina populations of *M. arenaria* race 2 and *M. incognita* race 3, and could be a useful rotation crop for tobacco (Johnson, 1998). In fact, sorghum supported minimum reproduction of *M. arenaria* race 2, *M. incognita* race 3 and *M. javanica* (Fortnum *et al.*, 2001b). Rotation with cotton or groundnuts reduced initial densities of *M. javanica* in Zimbabwe (Shepherd and Barker, 1990), but race 2 of *M. arenaria* predominated over *M. incognita* when cotton, maize, sorghum or rye preceded tobacco in South Carolina trials (Fortnum *et al.*, 2001b). Populations of *M. arenaria* race 1 can increase on groundnuts to levels that will cause moderate damage to tobacco (Hirunsalee *et al.*, 1995a). Where vegetable crops highly susceptible to *Meloidogyne* spp. are grown, particularly in subsistence agriculture, the damage to subsequent tobacco crops may be severe.

Most *Pratylenchus* species have a wide host range, and this can cause problems in selecting rotation crops. Lesion nematode populations can increase on bluegrass (*Poa* spp.), maize (*Z. mays*), rye (*Secale cereale*) and many legumes, but barley (*Hordeum vulgare*), oat (*Avena sativa*) and sweet potato (*Ipomoea batatas*) limit reproduction of some *Pratylenchus* species (Johnson, 1998). Rotating tobacco with marigold (*Tagetes* spp.) reduced *P. penetrans* in Ontario, Canada, below the economic threshold for 3 years (Reynolds *et al.*, 2000). Work in both Ontario and Quebec also found rotating tobacco with forage pearl millet to be highly effective in reducing *P. penetrans* (Jagdale *et al.*, 2000; Bélair *et al.*, 2002). The cyst nematodes and *D. dipsaci* have very limited host ranges, which facilitate their control by rotation, but their highly effective survival mechanisms may require the use of non-host crops over long periods. However, the use of tomato or resistant tobacco as a trap crop

reduced densities of *G. t. tabacum* by 64–84% (LaMondia, 1996b). Planting a trap crop after harvest and prior to seeding a rotation or cover crop was suggested as a practical and effective method to reduce TCN densities.

Physical control

Many of the early attempts to control nematode pests of tobacco, particularly in seedbeds or nurseries, relied on heating the soil either by burning grass and brushwood on the surface or by steaming the soil under a cover. Even though burning was recommended, it was realized that heat penetration was not always enough to kill nematodes at depths below 150 mm (Shepherd and Barker, 1990). However, subsistence farmers continue to use burning – or rabbing, as it is called in India – as their only method of seedbed control. A split-furrow rabbing method that involved burning husks from tobacco seed, pearl millet or wheat straw and tobacco stalks provided good control of *M. incognita*, *M. javanica* and various weeds in bidi tobacco seedbeds (Patel *et al.*, 1993). Steaming can kill weeds, nematodes, insects and fungal pathogens, but upsets the balance of soil bacteria similar to fumigation under some conditions, possibly leading to increases in soil ammonium and manganese toxicity. Effective penetration is usually about 300 mm but, being slow and expensive, the method is only suitable for seedbeds.

Research results with soil solarization have been variable and very sensitive to environmental factors, which are very hard to control, prompting suggestions that this method may not be practical for commercial-scale agriculture (Noling and Becker, 1994). Reports from Cuba, India, Italy and Tanzania, however, indicate that soil solarization can suppress nematodes in tobacco seedbeds long enough to produce usable transplants (Patel *et al.*, 1995a,b, 2001; Johnson, 1998). Solarization for a minimum of 15 days (and preferably for 40 days or longer) may provide an economic and practical method for small-scale farmers to reduce nematode parasitism significantly on tobacco transplants (Iglesias *et al.*, 1998; Hussaini *et al.*, 2001; Ravindra *et al.*, 2001). Combining solarization with other approaches may improve results (Zasada *et al.*, 2010). Control of *M. incognita*, *M. javanica*, *R. reniformis* and *T. vulgaris* from soil solarization,

alone or combined with other management practices, has been reported as similar to that from contact nematicides (Johnson *et al.*, 2005). Nematode densities rebounded more quickly in solarized soil (and in that treated with contact nematicides) than in soil treated with dazomet (Johnson *et al.*, 2005). Solarization may also change soil properties in tobacco seedbeds, and such effects should be accounted for (Johnson *et al.*, 2005).

Flooding has been associated with a degree of nematode control in places where the tobacco fields are flooded naturally or where tobacco is grown after paddy rice (Shepherd and Barker, 1990). Seventy-five days were needed to reduce the root knot populations by flooding, and some nematodes survived for up to 105 days.

Anaerobic soil disinfestation (ASD) may represent a more environmentally friendly method to reduce population densities of plant parasitic nematodes in tobacco fields (Lamers *et al.*, 2010). ASD involves creating and maintaining anaerobic conditions in saturated soil that contains a large volume of a uniformly incorporated carbon source (such as cover crop residues) (Lamers *et al.*, 2010). Treated soil is sealed (usually via a plastic mulch) to maintain low oxygen conditions in soil for approximately 6 weeks (Lamers *et al.*, 2010). Arugula (*Eruca sativa*), cowpea (*Vigna unguiculata*) and sorghum-sudangrass (*Sorghum × drummondii*) have been reported to be potentially useful cover crops for use with ASD to control *M. arenaria*, *M. incognita* and *M. javanica* (Kokalis-Burelle *et al.*, 2013).

Growers in regions without practical and effective root knot control options for transplant production have been advised to cut off as much of the galled root as possible before planting. This practice does not provide control, but does reduce initial nematode parasitism, hindering increase of the nematode population while plants are growing in the field (Johnson *et al.*, 2005).

Resistance

Virtually all flue-cured tobacco cultivars currently planted in the USA are resistant to races 1 and 3 of *M. incognita*, and this resistance has been incorporated into a large number of tobacco cultivars grown throughout the world (Johnson, 1989, 2017). Although root exudates of resistant tobacco cultivars may have nematocidal properties, resistance operates by preventing the

successful establishment of a feeding site rather than by inhibiting penetration (Shukla *et al.*, 1988; Schneider, 1991). Considerable variability has been reported in the reactions of specific root knot-resistant cultivars to various *Meloidogyne* species, and even populations, but resistance to races 1 and 3 of *M. incognita* and host race 1 of *M. arenaria* was found to be conditioned by the same gene (Ng'ambi *et al.*, 1999a). Cultivars possessing this gene have also been found to possess limited resistance to race 2 of *M. arenaria*, slight resistance to *M. javanica*, and more tolerance to attack from *Meloidogyne* species in general (di Vito *et al.*, 1998; Johnson, 1998; Ng'ambi *et al.*, 1999b). Currently, all LK cultivars in the Republic of South Africa (RSA) have resistance to *M. incognita* races 1 and 3, and although the RSA is supposed to have only races 2 and 4 of *M. incognita*, this resistance is holding up fairly well (A. Scholtz, Nelspruit, South Africa, personal communication). The gene responsible for resistance to races 1 and 3 of *M. incognita* (*Rk*) was apparently transferred into cultivated tobacco from *N. tomentosa*, and has been mapped to chromosome G (Yi *et al.*, 1998; Julio *et al.*, 2015). Several greenhouse studies indicated that prior infection with *M. arenaria* or *M. hapla* reduced the effectiveness of the *Rk* gene, although prior infection with other species of *Meloidogyne* did not reduce resistance to *M. javanica* in Zimbabwe (Shepherd and Barker, 1990; Johnson, 1998). Infection by mixed *Meloidogyne* species did not increase total nematode parasitism of *M. incognita*-resistant cultivars (Ng'ambi *et al.*, 1995). Resistance due to the *Rk* gene was unaffected by *M. arenaria* race 2 infection across temperatures ranging from 25 to 35°C. Greenhouse and field experiments also indicated that parasitism by race 2 of *M. arenaria* did not influence the reaction of tobacco roots to race 3 of *M. incognita* (Baum *et al.*, 1995a,b). Breeding lines resistant to race 3 of *M. incognita* have also been obtained from a *N. repanda* × *N. tabacum* cross, and to races 1 and 4 from accessions of *Nicotiana otophora* (Johnson, 1998).

Resistance to *M. javanica* has been found in *Nicotiana longiflora*, *Nicotiana megalosiphon* and *N. repanda*, as well as several other sources (Johnson, 1998). Zimbabwean burley cultivars Banket BRK1 through Banket BRK7 carry resistance to *M. javanica* resulting from two genes, the 'R' gene transferred from *N. repanda* and the 'L' gene from *N. longiflora* (Jack, 2010). Root knot-resistant

burley tobacco hybrids are also being developed in Malawi (Chamango *et al.*, 2015). Resistance to *M. javanica* was also discovered in 1950 in *N. tabacum* plants growing along the Zambezi River in Zimbabwe, in soil heavily infested by *M. javanica* (Schweppenhauser, 1975; Mackenzie *et al.*, 1986; Ternouth *et al.*, 1986). This resistance was initially thought to result from multiple genetic factors, but was later concluded to result from a single dominant gene ('T', now referred to as *Rk2* in the USA), perhaps involving modifying factors (Schweppenhauser, 1975). The partial resistance to *M. javanica* in plant selections containing *Rk2* was greater than that conferred by *Rk* (often now referred to as *Rk1*), and was very high when both genes were present together, at least in fields with low to moderate infestations of *M. javanica* (Ternouth *et al.*, 1986; Shepherd and Barker, 1990; Jack, 1996). More recent work observed that *Rk2* did not reduce root galling by a variant of *M. incognita* race 3, but often suppressed reproduction (Pollok *et al.*, 2016). Leaf morphology and agronomic traits were improved by crossing selections possessing *Rk2* with cultivated tobacco entries to develop the breeding line RKT15-1-1 (Mackenzie *et al.*, 1986), which was subsequently crossed with commercial tobacco cultivars to create germplasm (STNCA and STNCB) possessing both root knot-resistant genes (Ternouth, *et al.*, 1986). 'Better breeding lines' containing *Rk1Rk2* were reported to reduce root penetration rather than feeding site development (Shepherd, 1982). The STNC breeding lines have been used to incorporate resistance to *M. javanica* into multiple flue-cured tobacco cultivars in Zimbabwe (Kutsaga RK lines) and other countries in Africa. Resistance is currently being incorporated into other tobacco cultivars and tobacco types in multiple breeding programmes around the world, resulting in cultivars with increased resistance to multiple root knot biotypes (Pontes *et al.*, 2005; Mudzengerere and Shava, 2014; Verrier and Mazeau, 2016). The reactions of specific *Meloidogyne* species and races to this resistance has not been described thoroughly, and the mechanisms of resistance remain unknown (Pollok *et al.*, 2016). Unfortunately, resistance to *M. incognita* and *M. javanica* can break down at temperatures of 30–35°C, and some strains of *M. javanica* have reportedly parasitized resistant cultivars (Shepherd and Barker, 1990; Pollok, 2015).

Reduced reproduction of *M. arenaria* has been noted on selections of *Nicotiana knightiana*, *Nicotiana sanderae* and *Nicotiana velutina*, and by both *M. arenaria* and *M. javanica* on *Nicotiana glauca*, *N. longiflora*, *Nicotiana nudicaulis*, *Nicotiana plumbaginifolia* and *N. repanda* (Johnson, 1998). Six tobacco breeding lines were resistant to a North Carolina population of *M. arenaria* race 2 that may be useful in breeding programmes (Ng'ambi *et al.*, 1999b). Resistance to *M. arenaria*, races 1 and 3 of *M. incognita*, and *G. tabacum* has been incorporated into cultivated tobacco germplasm (Johnson *et al.*, 2005; Pollok *et al.*, 2015; Cogar *et al.*, 2017; Johnson, 2017).

Pleiotropic effects of root knot nematode resistance genes have been reported. Unfortunately, a severe vascular necrosis in response to infection by the M^SN^R strain of Potato virus Y (PVY-M^SN^R) appears to be a pleiotropic effect of the *Rk* gene (Rufty *et al.*, 1983a,b). However, resistance to PVY seems to be conditioned by a recessive gene epistatic to the *Rk* gene, and although *N. tomentosa* was the source of the *Rk* gene, accession 58 of that species was also found to be immune to the virus (Rufty *et al.*, 1983b).

Research at the molecular level is increasing our understanding of nematode feeding site establishment in tobacco, and may enable the development of more effective and durable resistance to nematodes, particularly to *Meloidogyne* species (Opperman and Conkling, 1994). This research has shown that the expression of many host genes changes as nematodes attempt to establish their feeding sites (Goddijn *et al.*, 1993). Tobacco plants engineered to express genes constitutively to produce glutamate decarboxylase (GAD) appeared to confer resistance to *M. hapla* (McLean *et al.*, 2003). The identification of host genes expressed only at nematode infection sites (such as *TobRB7*) could be used to target proteins inhibitory or toxic to nematode feeding structures (giant cells) only when and where they would be needed (Opperman *et al.*, 1994).

Nicotiana paniculata, *Nicotiana glutinosa*, *N. longiflora*, *N. plumbaginifolia*, *Nicotiana cordifolia*, *Nicotiana miersii*, *Nicotiana alata*, *N. repanda* and *Nicotiana noctiflora* are resistant to *G. t. solanacearum*, as are several tobacco introductions (Shepherd and Barker, 1990; Herrero *et al.*, 1996; Hayes *et al.*, 1997). Suppressed reproduction by

G. t. solanacearum has been noted in tobacco cultivars originally developed for resistance to wildfire disease (*Pseudomonas syringae* pv. *tabaci*), TMV and tobacco black shank (*P. nicotianae*). Wildfire resistance was incorporated into cultivated tobacco from *N. longiflora* and has been linked with resistance to *G. t. solanacearum* (Hayes *et al.*, 1997; LaMondia, 2002b). The TMV resistance and *G. t. solanacearum* resistance in flue-cured tobacco cv. NC 567 was obtained from *N. glutinosa* (Holmes, 1938). Suppressed reproduction by *G. t. solanacearum* has also been linked with the *Ph_p* gene from *N. plumbaginifolia* for resistance to race 0 of *P. nicotianae*, the causal agent of tobacco black shank (Johnson *et al.*, 2009). Widespread planting of cultivars possessing the *Ph_p* gene quickly shifted the race structure within populations of *P. nicotianae*, resulting in increased disease losses, illustrating the point that knowledge of resistance linkages is crucial to deploying resistant cultivars appropriately to the full range of pathogens and pests important in different tobacco production regions around the world (Parkunan *et al.*, 2010). Resistance against *G. t. solanacearum* has also been found to be effective against *G. t. tabacum* (LaMondia, 1988, 2002b). Although resistance to *G. t. solanacearum* from *N. longiflora* was reported to be multigenic (Spasoff *et al.*, 1971; Miller *et al.*, 1972; Crowder *et al.*, 2003), resistance to *G. t. tabacum* from the same sources, and from *N. plumbaginifolia*, appears to be conditioned by a single dominant gene(s) (LaMondia, 1991, 2002b; Crowder *et al.*, 2003).

Although host root exudates stimulate hatching of TCNs, hatching and penetration are similar for resistant and susceptible cultivars (LaMondia, 1988, 1995a; Wang *et al.*, 1997, 2001). Syncytia induced by TCN parasitism disrupt phloem function in tobacco roots, and resistance operates by inhibiting nematode feeding site establishment and possibly subsequent nematode development, remaining effective at 30°C, in contrast to the *Rk* gene for root knot resistance (Wang *et al.*, 2001; Doucet *et al.*, 2004). Early work associated resistance to *G. t. solanacearum* with severe root necrosis and stunting, but later research found no such association (Wang *et al.*, 1999). The effects of *G. t. solanacearum* on root size were similar on a resistant and a susceptible cultivar, but the increased parasitism in roots of

the susceptible cultivar caused greater losses in leaf weight. Crop rotation and nematicides can be used with resistant cultivars to reduce initial root damage and increase yields (Johnson *et al.*, 1989; Johnson, 1990; LaMondia, 2002b). Although intraspecific variability exists within *G. t. solanacearum*, nematode biotypes with increased reproductive ability on resistant cultivars have not been detected (Elliott *et al.*, 1986; Rideout *et al.*, 2000b; Syracuse *et al.*, 2004). The durability of resistance genes against the cyst nematodes *G. pallida*, *G. tabacum* and *Heterodera schachtii* may be associated with dispersal of the nematode, which can be influenced by crop production practices such as ploughing and harvest, and the number of nematode generations per year (Montarry *et al.*, 2015).

Chemical control

Nematicides remain an important tool in many tobacco production areas, despite the widespread use of nematode-resistant cultivars, particularly for root knot control. Some nematicides are no longer available due to concerns about detrimental effects to the environment, but others have been lost due to problems with continued effectiveness or concerns about applicator and environmental safety.

Tobacco transplants are now produced in greenhouse or 'float-bed' hydroponic systems in the USA and many other countries, and this practice eliminates the need for a nematicide in transplant production. However, outdoor tobacco seedbeds are still used by small-scale farmers in some countries. Methyl bromide was the most widely used seedbed fumigant because of its excellent broad-spectrum pest control and ease of use, but is no longer available (Shepherd and Barker, 1990). Metam sodium alone or combined with 1,3-dichloropropene (1,3-D) plus chloropicrin provided good disease, nematode and weed control in outdoor seedbeds in the USA (Csinos *et al.*, 1997, 2000; Makunde *et al.*, 2014). Metam sodium is being used for seedbed soil disinfestation in Zimbabwe, along with ethylene dibromide (EDB) and 1,3-D (Chinheya, Tobacco Research Board, Kutsaga, Zimbabwe, 2016, personal communication). The application of cadusafos, carbofuran and fenamiphos reduced parasitism by *M. incognita* and improved

plant growth in outdoor seedbeds in India (Swathi *et al.*, 1998; Gowda, 1999), but the use of dazomet provided similar or better control of *Meloidogyne* spp. than fenamiphos (Ramakrishnan *et al.*, 1999b). Fluopyram and a high rate of oxamyl controlled root knot in tobacco seed bed trials in Zimbabwe (Makunde *et al.*, 2014). Of course, no disease or weed control would be expected from the non-fumigant nematicides.

Tobacco fields are commonly fumigated for disease and nematode control in tobacco production areas such as the USA and southern Africa, but fewer soil fumigants remain available and increasing regulation is often required in order to use those registered for tobacco use. The products 1,3-D and chloropicrin are the primary soil fumigants applied in tobacco fields in the USA, where fumigation often targets nematode-disease complexes, such as bacterial wilt (*R. solanacearum*), and involves the application of both compounds as a row treatment applied 2–3 weeks before transplanting (Johnson, 2017). Broadcast fumigation is less common and more expensive, but is effective and can be performed as early as the previous autumn (Fortnum and Pullen, 2001). Canadian research on fumigation to control *P. penetrans* has shown that soil fumigants influence microbial activity in the soil, particularly that of nitrifying bacteria, early in the growing season (Tu *et al.*, 1995a,b, 1996). These effects generally dissipate by mid-season but, under certain environmental conditions (particularly prolonged cold, wet weather), early inhibition of soil nitrification can increase total alkaloids and decrease reducing sugars in cured leaf, reducing tobacco quality (Shepherd and Barker, 1990). Metam sodium can be applied similarly for nematode management, and has been used to control *G. t. solanacearum* in sandy loam soils in Virginia, USA, for lesion nematode control in Canada and for root knot control in Zimbabwe (Anon., 1979; Johnson and Wilkinson, 2002; Johnson, 2007, 2016, 2017; Makunde *et al.*, 2014). Application methods need to be optimized in Zimbabwe, where results have been inconsistent, especially in heavier soils (Anon., 1979; Makunde *et al.*, 2014a). High rates of allyl isothiocyanate, a compound related to metam sodium that was described as a 'biofumigant' by the US Environmental Protection Agency, has also been observed to control *G. t. solanacearum*

in several field studies (Darnell and Johnson, 2016a,b; Cogar and Johnson, 2017). Research for other crops on 'site specific' nematode management, i.e. fumigating soil only in areas within an infested field where nematode densities are above threshold levels, may hold promise for use in tobacco to reduce fumigant usage (Liu *et al.*, 2014).

Until recently, the use of non-fumigant nematicides had dropped significantly, due to increased regulatory restrictions, improved fumigation technology and wider recognition of nematode disease complexes. In addition, annual use of some nematicides was linked with reduced effectiveness, probably due to enhanced biodegradation (Davis *et al.*, 1993). Non-fumigant organophosphate and carbamate nematicides do not reduce nematodes as effectively as fumigants (Fortnum *et al.*, 2001a,b; Johnson *et al.*, 2005). However, they may be used more extensively in the future as fumigant nematicides become less available (H. Papenfus, ACAC, CORESTA, 2016, personal communication). In Zimbabwe, and probably southern Africa, non-fumigant nematicide use has increased due to the high cost of fumigants and lack of adequate application methods for small-scale farmers, who make up about 80% of the tobacco growers. Fenamiphos has been recommended as a pre-plant nematicide in Malawi, and fenamiphos and oxamyl have each been used as supplements to fumigation in southern Africa, to extend the period of control when nematode densities are high or where there are poor growing conditions early in the season (Johnson, 1998). Fluopyram is registered and recommended for tobacco nematode control in a number of African countries, including Malawi, Zambia and Zimbabwe (H. Papenfus, ACAC, CORESTA, 2016, personal communication). Positive results have also been obtained from transplant water or in-furrow application of fluopyram for the control of *G. t. solanacearum* (Darnell and Johnson, 2016a,b; Cogar and Johnson, 2017). Oxamyl effectively reduces initial densities of tobacco cyst nematodes (LaMondia, 1996a; Darnell and Johnson, 2016a,b). While fosthiazate provided good to excellent control of *M. arenaria*, *M. incognita* and *G. t. solanacearum*, it has never been registered in the USA (Johnson, 1995; Pullen and Fortnum, 1999). Fluensulfone was registered in the USA for tobacco nematode control in 2016, but

results have not been consistent in US and Canadian trials (Csinos *et al.*, 2010, 2012, 2016; Saude *et al.*, 2016). The application of a colloidal suspension of propylene glycol alginate in the transplant water and one month after transplanting reduced soil densities of *G. t. tabacum* and *M. arenaria* (Navas *et al.*, 2007).

Biological control

Research continues to identify practical and effective biological methods for controlling plant parasitic nematodes on tobacco. Results from Zimbabwe and the USA using *Paecilomyces lilacinus* (now *Purpureocillium lilacinum*), *Bacillus* spp. and *Pasteuria penetrans* to control *Meloidogyne* spp. on tobacco have not been encouraging, although some suppression of root knot nematodes was observed and a commercial product (PL plus) was registered in the RSA (Weibelzahl-Fulton *et al.*, 1996; Johnson, 1998; Johnson *et al.*, 2005). Screening experiments continue to evaluate bacterial and fungal species that are effective in reducing nematode parasitism of tobacco (Subhashini *et al.*, 2009; Lin *et al.*, 2012; Chen *et al.*, 2015). A biological nematicide containing dried fermentation solids and solubles from *Myrothecium verrucaria* strain AARC-0255 reduced *M. incognita* and increased plant growth, but did not perform as well in trials against *G. t. solanacearum* (Johnson *et al.*, 2005).

Acibenzolar-S-methyl (ASM) induced systemic acquired resistance (SAR) in tobacco, and reduced parasitism of flue-cured and oriental tobacco by *G. t. solanacearum* was observed under greenhouse conditions, but also often induced deleterious plant growth effects (Parkunan *et al.*, 2009a). The effects of ASM on reproduction by *G. t. solanacearum* were only consistent on resistant cultivars possessing *Ph_p* (Parkunan *et al.*, 2009b). The incorporation of a combination of the plant growth-promoting rhizobacteria (PGPR) into soil before transplanting suppressed reproduction by *G. t. solanacearum* without undesirable effects. Similar application of a combination of the PGPR consistently reduced nematode development and reproduction of *G. t. solanacearum* on cultivars with and without the resistance gene *Ph_p*, but similar reductions associated with foliar application of ASM were less consistent. Combinations of chitosan and PGPR strains promoted growth of tomato, cucumber, pepper and

tobacco, and reduced galling by *Meloidogyne* by similar mechanisms (Kloepper *et al.*, 2004). Mutualistic endophytic fungi have been used to increase resistance in plants to nematodes by adding the antagonistic strains to the transplant production systems (Hallmann *et al.*, 2001). Using this system of targeted application could reduce markedly the costs involved in using biological control.

Plant residues and by-products are also being evaluated as materials to reduce nematode population densities. Essential oils from the leaves of *N. tabacum* and aqueous extracts from the leaves of *Azadirachta indica*, *Melia azedarach* and other plants have shown nematocidal properties against *M. incognita* (Johnson, 1998; Ramakrishnan *et al.*, 1999a). Positive results have been noted using a natural oil extracted from sesame to control *M. javanica*, but not with cinnamon extract to control *G. t. solanacearum* (Makunde *et al.*, 2014; Darnell and Johnson, 2016a).

The term 'biofumigation' has been coined to describe the use of plant residues (such as the brassicas) to generate nematocidal compounds (such as isothiocyanate) in soil (Matthiessen and Kirkegaard, 2006; Zasada *et al.*, 2010). Although positive results have been observed, activity may vary widely based on numerous factors (Matthiessen and Kirkegaard, 2006; Zasada *et al.*, 2010). *Brassica juncea* (L.) Czern. is apparently a good host of *M. incognita* and *M. javanica*, and so may be a poor cover crop choice to reduce these nematodes, but the incorporation of *B. juncea* seed meal reduced densities of these nematodes in laboratory and greenhouse assays (Zasada *et al.*, 2009; Rahman *et al.*, 2011; Edwards and Ploeg, 2014). Lesion nematode species may be less sensitive to brassicaceous seed meals than *M. incognita* (Zasada *et al.*, 2009; Brammall *et al.*, 2014). *G. t. solanacearum* densities tended to be lower and flue-cured tobacco yields tended to be higher when *B. juncea* seed meal was incorporated into soil before transplanting, but these trends were never statistically significant (Table 19.2; C.S. Johnson, unpublished data). The incorporation of sunn hemp (*Crotalaria juncea*) or *Sesbania bispinosa* residue suppressed nematodes for 70 days after seeding in bidi tobacco seedbeds in India (Patel and Patel, 1999). Incorporating garlic pellets and sesamin oil in the soil is also being evaluated as a possible management option for root knot management in Zimbabwe. Pre-plant

incorporation of cottonseed meal-based fertilizer or chitin-urea amendments failed to reduce densities of *G. t. tabacum* in shade tobacco fields and influenced quality characteristics of the crop adversely, but the use of jatropha seed cake as an organic basal fertilizer reduced root knot galling and increased the yield and quality of flue-cured tobacco in Malawi (LaMondia, 1994; Mtonga *et al.*, 2015). Organic manures can limit parasitism by *Meloidogyne* spp. and increase tobacco yields, but also have the potential for significant phytotoxicity (Johnson, 1998).

Summary of management measures

Consistently reliable nematode control in tobacco requires the use of multiple approaches. Even in areas where nematicide use is routinely necessary, satisfactory control of tobacco nematodes is based on sound crop rotation plans, when available, planting resistant cultivars when available, and the destruction of tobacco roots and stalks as soon after harvest as possible.

The use of healthy transplants is critical to achieving satisfactory tobacco yield and quality. Greenhouse seedling production provides such plants, but when transplants are produced in outdoor seedbeds, these seedbeds should be fumigated to minimize nematode parasitism. Tobacco seedbed locations should be rotated, and nematode-resistant crops should be planted in seedbed areas. Tobacco should only be grown in specific fields for one or two consecutive growing seasons before rotation to other crops that either are non-hosts to the nematode species present or possess resistance. Grass crops are often preferred for rotation with tobacco.

Resistant cultivars are widely available for *M. incognita* races 1 and 3, and have shown at least partial resistance or tolerance to other races and species of *Meloidogyne*. Partial resistance is now also increasingly available to other root knot nematode species, especially *M. javanica* and *M. arenaria* in some areas. Resistance to the TCNs is also available in a number of cultivars now being planted around the world, and cultivars are available with resistance to multiple root knot species in addition to TCNs (Johnson, 2017). In many situations, the use of good rotations and a resistant cultivar may be sufficient to limit crop

Table 19.2. Effect of mustard (*Brassica juncea*) seed meal versus standard nematicides on final populations of *Globodera tabacum solanacearum* (tobacco cyst nematode – TCN) and flue-cured tobacco yield.

Nematicide	Rate/ha	Application method ^a	Final TCN eggs/500 cm ³ soil			Cured leaf yield (kg/ha)		
			2010	2011	2012	2010	2011	2012
1,3-D	86 l	Fum-Row	10,228 b	30,544 a	24,291 a	3263 a	4248 a	4071 a
1,3-D + chloropicrin	101 l	Fum-Row	11,369 b	–	–	3555 a	–	–
Metam sodium	234 l	Fum-Row	10,888 b	–	–	3602 a	–	–
Oxamyl	5 l	Bcast-PPI	–	21,125 a	27,969 a	–	3957 a	3981 ab
Mustard seed meal	280 kg	Bcast-PPI	12,298 b	28,298 a	–	3125a	3870 a	–
Mustard seed meal	560 kg	Bcast-PPI	16,296 ab	22,059 a	–	3343 a	4022 a	–
Mustard seed meal	841 kg	Bcast-PPI	10,528 b	17,819 a	–	3175 a	4039 a	–
Mustard seed meal	1345 kg	Bcast-PPI	12,200 b	19,901 a	–	3508 a	3890 a	–
Mustard seed meal	1681 kg	Bcast-PPI	–	–	33,129 a	–	–	3686 a–e
Mustard seed meal	1962 kg	Bcast-PPI	–	–	24,209 a	–	–	3951 ab
Mustard seed meal	2242 kg	Bcast-PPI	–	–	23,543 a	–	–	3848 abc
Untreated control	–	–	16,358 ab	26,732 a	33,669 a	3194 a	3688 a	3551 b–f

Notes: Data presented are non-transformed means of four replications, but statistical analysis was performed on transformed ($\log_{10} [x + 1]$) data. Means followed by the same letter(s) are not significantly different based on the Waller–Duncan *k*-ratio *t*-test (*k*-ratio = 100). ^aFum-row = row versus broadcast fumigation at bedding; Bcast-PPI = broadcast spray, incorporated, prior to transplanting.

losses to nematodes, but nematicide application will often help tobacco to realize its full yield potential. If the nematode density is high, a fumigant will tend to provide better control than a non-fumigant nematicide; multi-purpose fumigants are also necessary in fields with a history of nematode–disease complexes.

Methods of diagnosis

The selection of appropriate nematode control measures for tobacco depends on accurate assessment of the nematode density(ies) present in fields at transplanting. Bioassays or nematode extraction from soil and/or root samples can be used to detect population densities of important nematode parasites of tobacco. Since bioassays can be quite sensitive compared with nematode from soil extraction, bioassays may be the preferred method for monitoring nematode densities where *Meloidogyne*, *Globodera* or *Ditylenchus* species are the predominant parasitic nematode. Bioassays must, however, be performed well ahead of time because several weeks will be required for any galling or cyst production to become evident. Visual assessment of tobacco roots ploughed out of the soil at the end of the growing season is commonly recommended to US tobacco producers (Johnson, 2017).

Reliable quantitative estimates of nematode densities are necessary to optimize nematode management. Nematode assay and advisory services are available in some of the tobacco-growing areas of North America, Europe and elsewhere, but are not found in many other areas. Most tobacco growers in the USA do not assay their fields for nematodes every year (Johnson, 1989). However, periodic nematode assays can be used with field histories to estimate the species composition and relative damage potential of nematode densities in tobacco fields (Peterson, 2015). Nematode assays from soil are rarely able to differentiate nematodes beyond the genus level, even when such distinctions are important criteria for nematode management decisions. The integration of classic morphological techniques with enzyme phenotyping and with more accurate, reliable and practical molecular methods are increasing our ability to identify rapidly and reliably the nematode species present in fields (Nakhla *et al.*, 2010; de Araújo Filho *et al.*, 2016; Pollok *et al.*, 2016). Some of these techniques are outlined in Chapter 2, this volume.

Conclusions and Future Prospects

Significant progress has been made in developing tools for tobacco farmers to reduce the impact of

plant parasitic nematodes on tobacco production. In some areas, nematode problems that were once significant are now considered minor problems. However, nematodes are widely recognized as significant problems in other tobacco-producing regions, either those relatively new to tobacco production or where small landholders have replaced large-scale commercial farmers in producing the crop. Cultivars with nematode resistance are becoming increasingly available and, together with cultural practices such as crop rotation and early root and stalk destruction, have enabled growers in many parts of the world to reduce their dependence on nematicides, particularly in managing root knot and tobacco cyst nematodes. More effective and durable forms of resistance to plant parasitic nematodes may be available in tobacco cultivars as advances in our understanding at the molecular level enable the development of mechanisms to inhibit nematode penetration and development with minimal impact on plant growth.

Nematicide use, however, remains necessary in many situations and areas, particularly in southern Africa, where *M. javanica* is the

predominant nematode and a major problem. Research continues to identify effective biological agents that tobacco producers can use to protect their crop, but the need remains for new nematode management agents (NMAs) that can be used, in the short term, whether biological or not. Fortunately, new synthetic NMAs are being developed and registered that, while not as effective as soil fumigants, still offer practical solutions for growers and with lowered risk of injury to applicators, agricultural workers and the environment. Plant parasitic nematodes remain an important production constraint to tobacco farmers in many areas of the world, but significant progress has been made, with good potential for further progress in the future.

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20 Nematode Parasites of Pineapple*

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The cultivated pineapple *Ananas comosus* var. *comosus* (Bromeliaceae) is a monocotyledonous perennial herb that probably originated in South America (Collins, 1968; Coppens d'Eeckenbrugge and Leal, 2003). Cultivar clones are often specific to a growing area but are all classified as *A. comosus comosus*, the edible pineapple. In South America, the fruits of some wild species (e.g. *Bromeiia karatas* 'pinuella') are eaten, whereas others (*Ananas comosus* var. *erectifolius*) are used as fibre crops (Py *et al.*, 1984; Coppens d'Eeckenbrugge and Leal, 2003).

Pineapple is grown through tropical and subtropical climates. Asia produces about 46% of world pineapple tonnage (FAOSTAT, 2015). The Philippines and Thailand concentrate on the canned commodity and are the largest producers and exporters in Asia. Central and South America account for about 31% of world pineapple production, with most production geared towards fresh fruit (FAOSTAT, 2015). Costa Rica and Brazil are the major pineapple producers accounting for 20% of world production. Africa produces about 12% of the world total, with most of the production originating in Nigeria. The main pineapple producers in the Pacific are Australia (Queensland) and Hawaii (FAOSTAT, 2015).

Cultivation techniques

Six major groups of vegetative pineapple clones are grown (Chan *et al.*, 2003). Commercial plantations plant low acid hybrid clones for fresh fruit and cultivate higher acid Cayenne clones for canning. Clones and hybrids have slightly different agronomic characteristics. Pest problems differ between the hybrids and traditional cultivars, and hybrid behaviour towards plant parasitic nematodes may be different. Smooth Cayenne predominates in large plantations producing canned pineapple throughout the world, and similar cultural practices are used for low acid and canning cultivars. Research on nematode diseases has been conducted primarily in the intensive production systems growing Smooth Cayenne.

Pineapple is cultivated for its 100–200 berry-like fruitlets arranged around a central core continuous with the peduncle (Collins, 1968). Cultivated pineapple is self-sterile and propagated vegetatively from crowns, slips, suckers or stumps (Evans *et al.*, 1988). Crowns removed at harvest from fruits intended for canning are used as planting material (seed). Slips originate axially and are borne on the fruit stalk. Slips are commonly used as seed in South Africa. Suckers begin growing at

*A revision of the chapter by B.S. Sipes, E.P. Caswell-Chen, J.-L. Sarah and W.J. Apt in the second edition.

floral differentiation, originating from axillary buds on the stem. Suckers may be removed from the plant after fruit harvest and used as seed or left on the plant to produce a ratoon crop.

Pineapple is planted throughout the year. Planting density ranges from 15,000 to 120,000 plants/ha in single- to triple-row beds, depending on the production system, ecological conditions and the clone. In a typical commercial production system with irrigation, seed is typically planted in two-row beds (rows 40–60 cm apart, beds 120–140 cm centre to centre) with densities of 50,000–75,000 plants/ha (Guyot *et al.*, 1974; Lacoëuilhe and Guyot, 1979; Py *et al.*, 1984; Evans *et al.*, 1988). Beds may be covered with mulch before planting, to retain fumigant and moisture, increase soil temperature and control weeds. A black plastic mulch is commonly used, but other biodegradable materials can be used, especially in organic production. A soil fumigant is usually injected (predominately 1,3-dichloropropene) for nematode control during soil preparation or as the mulch is being laid (see 'Management Measures').

Pineapple is essentially a xerophyte with stomata and trichomes adapted for reducing water loss, a growth habit allowing the collection of rainfall, and a crassulacean acid metabolism. Pineapple has retained epiphytic characteristics, such as the ability to absorb water and minerals through the leaves, and a fragile root system (Py *et al.*, 1984). Pineapple can be grown in areas with as little as 600 mm annual rainfall. The adventitious root system is not extensive, penetrating the soil to a depth of 5–60 cm and extending 40–80 cm horizontally from the base of the plant (Guérout, 1975); consequently, supplemental irrigation can improve plant growth and yield greatly. Nematode parasitism of the roots can affect yield severely. Although pineapple can survive poor growing conditions, high levels of nitrogen, potassium and some microelements, such as iron, are required for profitable yield. Pre-plant fertilizers are placed in the bed during soil preparation, helping to maintain pH in the optimum range of 4.5–5.5, whereas post-plant fertilizers are applied as foliar sprays or through drip irrigation.

Ethylene or other growth regulators are used to force flowering ('forcing') 6–18 months after planting. The time of forcing depends on the climate, the seed material used, and the intended use of the fruit (canning or fresh market) (Py *et al.*,

1984). Fruit are ready for harvest approximately 5–9 months after forcing. If nematode problems are not severe and soil conditions are adequate, a second crop, the ratoon, can be harvested.

Nematodes of Pineapple

More than 100 species of plant parasitic nematodes have been associated with pineapple root systems. The most important species are the root knot nematodes *Meloidogyne javanica* and *Meloidogyne incognita*, the reniform nematode *Rotylenchulus reniformis*, and the root lesion nematode *Pratylenchus brachyurus*.

Other plant parasitic nematodes associated with pineapple are of limited or unknown pathogenicity. *Paratylenchus minutus* can be found in exceedingly high numbers in pineapple fields in Hawaii, with population densities of more than 5000/250 cm³ soil (Linford *et al.*, 1949; B.S. Sipes, unpublished), and has been associated with yield decline in Malaysia (Nik Masdek *et al.*, 2007). *Helicotylenchus* spp. are commonly associated with pineapple (Redondo and de Agudelo, 1992; Nath *et al.*, 1997; da Costa *et al.*, 1998; Quesada and Barboza, 1999; Daramola *et al.*, 2013a), and *Helicotylenchus dihystra* has been associated with damage to pineapple in glasshouse studies (Ko and Schmitt, 1993). In South Africa, *Helicotylenchus*, *Scutellonema* and *Rotylenchus* have been reported as problematic (Keetch and Purdon, 1979). *Aorolaimus* sp. has been associated with reddish leaf symptoms in Brazil (da Costa *et al.*, 1998). The association of a plant parasitic nematode with a plant does not prove that the nematode is damaging, but detection of such associations should stimulate research to determine possible damage.

Nematode parasitism should be suspected if symptoms of stress are evident in the foliage despite satisfactory climatic and agronomic conditions. In some cases, careful observation of the roots may permit the diagnosis of nematode infection, but nematode sampling is usually required to diagnose the nematode species involved.

Meloidogyne

M. javanica is a severe pathogen of pineapple. It is the most important pineapple nematode in

Australia, being widespread in south-east Queensland, and is a significant concern in Kenya, Mexico, South Africa, Thailand, Zimbabwe and some areas of the Philippines. *M. javanica* was the main nematode disease problem in Hawaiian pineapple from 1920 until the 1950s, when the reniform nematode became the primary challenge (Rohrbach and Apt, 1986).

M. incognita has been reported from several pineapple-growing areas, but does not cause serious damage, except in some areas of Puerto Rico and Mexico (Ayala *et al.*, 1969; Garcia-Espinosa and Adam, 1972; Devi, 2007; Daramola *et al.*, 2013a). In Côte d'Ivoire, *M. incognita* caused damage when some plantations were first established, but its importance has diminished relative to *P. brachyurus* (Guérout, 1965).

Symptoms of damage

Second-stage juveniles infect the primary root tips of pineapple. Root growth is retarded within 24 h of nematode penetration, and usually a terminal club-shaped gall is produced as the nematode develops (Godfrey and Oliveira, 1932) (Fig. 20.1). Large galls are not formed, but small, non-terminal fusiform galls may form and cause brooming of the root system (Godfrey, 1936). Second-generation juveniles infect lateral roots, causing a reduction of the total root length of the plant, decreased nitrogen absorption and plant growth rate, and reduced yield (Magistad and Oliveira, 1934; Godfrey and Hagan, 1937). Severe infections result in a stunted root system, poor anchorage and plants that are more susceptible to moisture and nutrient stress.

Biology and life cycle

The life cycle of *Meloidogyne* spp. is covered in Chapter 2, this volume. Population increase of *Meloidogyne* spp. on pineapple is slow compared with other host plants. Nematode population densities remain at pre-plant levels for several months after planting (Fig. 20.2) (Stirling and Nikulin, 1993). After these initially stable levels, the nematode population enters a linear growth phase, and over the next 6 months, reaches a plateau. The plateau population densities are maintained throughout the remainder of the crop cycle. Significant population decreases do not occur until the pineapple is destroyed and the field fallowed.

Pathotypes and races/biotypes

Distinct host responses by populations of *M. javanica* and *M. incognita* towards pineapple cultivars and clones have not been observed. The lack of anecdotal evidence from the field has not prompted research into the area. The absence of host-plant resistance to deploy in the field may contribute to this situation. The importance of genetic variation among populations of root knot nematodes should not be ignored in pineapple cultivation. Research to assess the genetic variation that exists among geographic isolates of root knot nematodes is needed to determine the appropriateness of comparing damage and management studies from different places.

Survival and dissemination

Juveniles of *M. javanica* may survive in desiccated soil without a host for 20–24 weeks, although

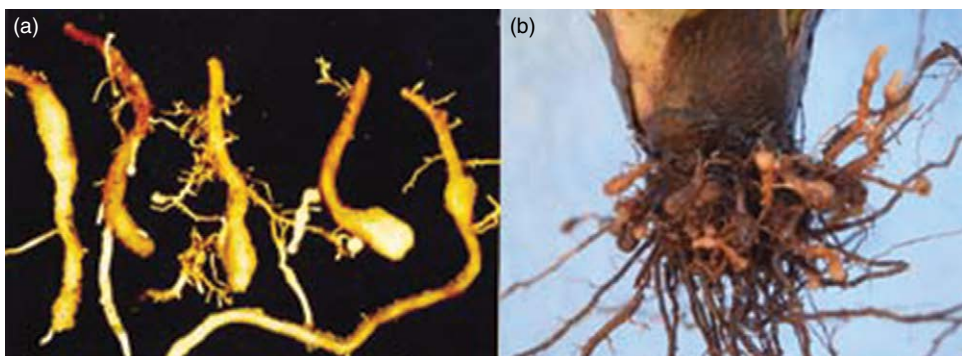


Fig. 20.1. Root clubbing on pineapple caused by *Meloidogyne javanica*. (From Sipes *et al.*, 2005.)

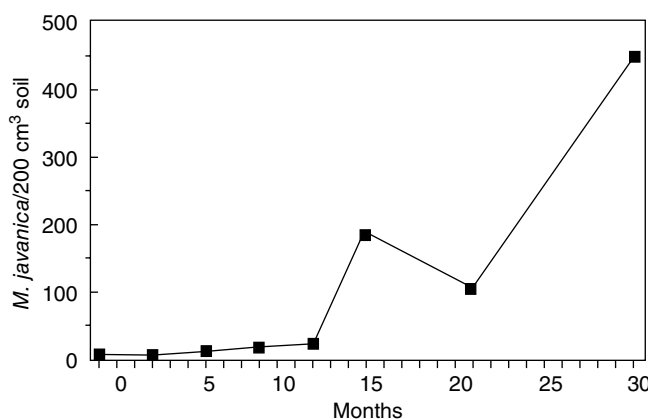


Fig. 20.2. Population increase of *Meloidogyne javanica* on pineapple in Australia.

soil moisture influences survival (Godfrey *et al.*, 1933; Towson and Apt, 1983). The time required to reduce the soil densities of *M. javanica* juveniles by 50% in Hawaiian soils was 3, 5, 110, 10 and 3 days at soil moistures of -0.16 , -0.30 , -1.1 , -15 and -92 bars, respectively (Towson and Apt, 1983). *M. javanica* can survive, although at low levels, as long as 2 years in fallow field soil (Godfrey, 1936).

The nematode survives a wide range of temperatures; however, 127 min at 40°C is lethal to juveniles, while 4.5 days at 40°C is lethal to eggs (Hoshino and Godfrey, 1933). Bare pineapple soils in Hawaii may reach 40°C at a depth of 0.6 cm during the summer and, if covered with mulch paper, temperatures greater than 40°C may extend to a depth of 7.5 cm (Hagan, 1933).

The spread of root knot between root systems of adjacent plants is quite slow. Godfrey (1936) observed that up to 7 months were required for an infestation to move 30 cm within a row. The root knot nematodes may be disseminated over long distances in soil adhering to workers' shoes, implements and equipment that is moved from field to field. In South Africa, the nematode is spread by planting infected stumps, so seed material from infested areas is destroyed.

Environmental factors affecting parasitism

The minimum temperature for infection by *M. javanica* is approximately 13°C (Godfrey, 1936). *M. javanica* is capable of surviving a wide range of pH levels, and can infect pineapple roots successfully at soil pH of 4.0–8.5, the range of pH at which pineapple is usually grown (Godfrey and

Hagan, 1933). In Hawaii, low soil pH may favour the reniform nematode over root knot nematode; however, low non-detectable populations of root knot nematodes survive and can resurge in a field if cultivation is switched to a different host.

Other hosts

M. javanica has a host range of more than 770 plants, including many economically important crops such as potato, tomato, grape and tobacco. This wide host range makes the selection of cover crops, green manures and intercycle crops very challenging.

Disease complexes

Galls of *M. javanica* are subject to secondary invasion by various fungi that cause blackening and drying of the nematode galls and death of the nematodes within the gall (Godfrey, 1936; Keetch, 1982). Pineapple plants infected with Pineapple mealybug wilt associated virus and *M. javanica* exhibited much more severe wilt symptoms on the shoot and significantly greater plant collapse and death of plants (de Freitas Ferreira *et al.*, 2015).

Economic importance and damage threshold

Godfrey (1936) suggested that plants could become well established when the density of root knot nematodes was less than approximately 6 juveniles/cm³ soil. Under South African conditions, a single

juvenile of *M. javanica* in a root or soil sample is interpreted as a potential problem (Keetch, 1982). In Australia, economically significant crop losses occur in the pineapple ratoon crop when nematode densities at 12 months after planting are greater than 1–5 juveniles/200 cm³ soil (Stirling and Kopittke, 2000). In Kenya, *M. javanica* appears to be the most damaging nematode (Kiriga, 2017).

Rotylenchulus reniformis

The reniform nematode *R. reniformis* occurs in the tropics, subtropics and warm temperate regions throughout the world. It is the major nematode problem of pineapple in Hawaii and the Philippines (Davide, 1988). Reniform nematode is also important in the Caribbean (e.g. Puerto Rico), in some areas of Thailand, in north Queensland, Australia, and in Oaxaca, Mexico. In South Africa, *Rotylenchulus parvus* is frequently observed, but is of little economic importance (Keetch, 1982).

Symptoms of damage

In Hawaii, the leaves of infected plants are less erect than those of healthy plants, are reddish in colour and show poor growth. The foliar symptoms are similar to those caused by nutrient or moisture stress. In contrast to the symptoms observed in root knot nematode infections, primary roots of pineapple infected with *R. reniformis* continue to elongate and provide good anchorage for the plant. However, reniform nematode infection inhibits secondary root formation, and root systems are poorly developed (Fig. 20.3). Heavy infections may result in plant collapse and death of the pineapple plant. Improper management of reniform nematodes typically leads to ratoon crop failures in Hawaii. Fewer ratoons are formed per mother plant, and the flowering of the ratoon plants is irregular, leading to fruit that matures too early or too late.

Biology and life cycle

The reniform nematode has a unique life cycle. Egg hatch is stimulated by root exudates of certain host plants (Kahn, 1985), and second-stage juveniles leave the egg and move into the soil.



Fig. 20.3. Pineapple root biomass from plants inoculated and not inoculated with *Rotylenchulus reniformis*. (From Sipes *et al.*, 2005.)

Once in the soil, the reniform nematodes undergo three moults without feeding, yielding adult males and ‘pre-parasitic’ females. Females enter the root system and initiate a feeding site. Females continue to develop, swell, and become sedentary; the mature egg-producing females deposit an average of 60 eggs into a gelatinous matrix (Linford and Oliveira, 1940; Bird, 1984). Although amphimixis appears to be the rule, some populations reproduce parthenogenetically (Nakasono, 1977, 1983).

Females induce their syncytium in the stele of the root (Robinson *et al.*, 1997). The syncytium is formed from a single endodermal cell that enlarges to incorporate additional cells in the pericycle, the vascular parenchyma and sometimes the phloem (Rebois *et al.*, 1975; Robinson *et al.*, 1997). Males do not appear to feed at any time.

The population dynamics of the reniform nematode in pineapple are similar to those of *M. javanica* in pineapple (Fig. 20.4). The reniform nematode population density does not increase immediately after the pineapples are planted and begin rooting. Nematode population densities remain at pre-plant levels for up to 8 months (Sipes and Schmitt, 1994b). After this period of relatively static population, it enters a linear growth phase and increases to levels of up to 10,000 nematodes/250 cm³ soil within 6 months (Sipes and Schmitt, 1994b). The reniform nematode population remains at these peak levels throughout the crop cycle, showing only slight decreases at the initiation of pineapple flowering. The delayed population development could be related to endogenous protease inhibitors found in the pineapple roots (Radovich *et al.*, 2009). The protease

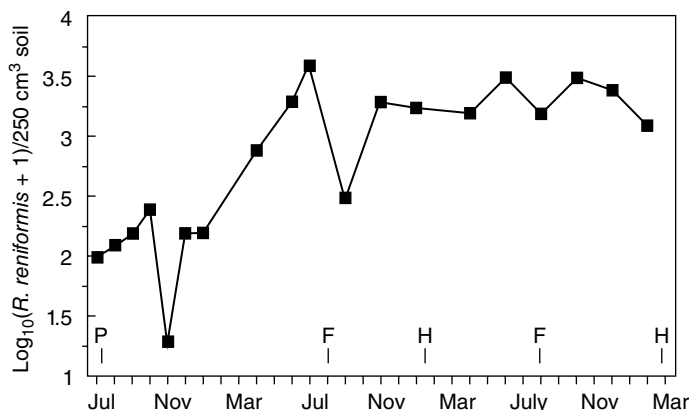


Fig. 20.4. Population increase of *Rotylenchulus reniformis* on pineapple in Hawaii. (From Sipes *et al.*, 2005.)
 Note: P = planting; F = flowering; H = harvest.

inhibitor concentration is greater in pineapple infected with reniform nematode than in uninfected plants, suggesting a systemic acquired resistance response to nematode infection by the pineapple plant (Chinnasri and Sipes, 2005).

Pathotypes and races/biotypes

Distinct races of the reniform nematode are not generally recognized. On the basis of host range and reproductive strategy, the existence of races has been suggested (Dasgupta and Seshadri, 1971; Heald, 1978; Nakasono, 1983; McGawley *et al.*, 2010). There are differences in temperature optima and reproductive behaviour among populations of reniform nematode (Nakasono, 1977, 1983). For example, exposure to low temperatures (15°C) resulted in decreased reproduction in populations from Puerto Rico compared with populations from Louisiana and Texas, USA (Heald and Inserra, 1988). DNA differences in the internal transcribed spacer (ITS) region and microsatellites show variation among populations, but this genetic variation has yet to be correlated with other biological, pathogenic, or parasitic characteristics (Agudelo *et al.*, 2005; Arias *et al.*, 2009).

Survival and dissemination

The reniform nematode tolerates extreme temperatures and survives extended periods without a host. Reniform nematode populations from Louisiana, Texas and Puerto Rico survived for

6 months without a host at temperatures of −5, −1, 4 and 25°C (Heald and Inserra, 1988). Although the reniform nematode is able to survive low soil moisture, soil moistures greater than 7% increase nematode survival at 25°C, but decrease nematode survival at temperatures below freezing (Heald and Inserra, 1988). *R. reniformis* can survive for 2 years in fallow soil. Apparently, the nematode survives fallow periods in the egg stage or as anhydrobiotic juvenile stages, depending on soil moisture (Apt, 1976; Tsai and Apt, 1979).

Environmental factors affecting parasitism

The optimum temperature for development of the reniform nematode is 25–29°C, and reproduction is limited by temperatures above 36°C (Rebois, 1973; Heald and Inserra, 1988). Soil temperatures in pineapple-growing regions are extremely favourable to the development of the reniform nematode.

The reniform nematode did not become a significant agronomic problem in Hawaii until the mid-1950s. The tendency of the pineapple industry to use shorter and shorter fallow periods is thought to have contributed to the increasing problem with reniform nematode (Rohrbach and Apt, 1986). In addition, the pH of pineapple soils decreased steadily from 1930 to 1950, due to the application of ammonium sulfate fertilizers. The pH in some fields in Hawaii was as low as 3.2 by 1950. The optimal pH

for reproduction of the reniform nematode in Hawaiian soils is approximately 4.8–5.2 (Rohrbach and Apt, 1986).

Another factor contributing to the increased importance of reniform nematode in Hawaiian pineapple production was soil fumigation. Fumigation began in the late 1940s. Soil fumigants undoubtedly suppressed nematode antagonists in the soil (Rohrbach and Apt, 1986). Fertilizer application and fumigation practices, combined with intensive monoculture, appear to have created a soil environment supportive of reniform nematode survival and reproduction. Consequently, in 35–40 years, *R. reniformis* progressed from an initial limited occurrence to a major limiting factor in Hawaiian pineapple culture.

Other hosts

The reniform nematode has an extensive host range that includes more than 300 plant species (Robinson *et al.*, 1997). Many weed species commonly found in pineapple- and sugarcane-growing areas are hosts to the reniform nematode (Linford and Yap, 1940; Birchfield and Brister, 1962). Many important crop species, such as soybean, cotton, pigeonpea and beans, are also hosts to the reniform nematode.

Economic importance and damage threshold

The reniform nematode is a serious and damaging pathogen of pineapple. In Hawaii, high population densities of the nematodes combined with moisture stress can result in complete ratoon failures (Rohrbach and Apt, 1986). D-leaf weight (the youngest mature leaf), plant height and root biomass did not differ ($P > 0.05$) among a range of reniform nematode populations at 6 or 12 months. However, plant crop fruit yield did differ among the initial population density ranges. In Peru, populations of *R. reniformis* were associated with decreased D-leaf length (Julca-Otiniano *et al.*, 2005). In pineapple, the D-leaf and plant height are highly correlated to final fruit weight. Pre-plant populations of *R. reniformis* below 300 nematodes/250 cm³ soil damage pineapple, but are not the major factor limiting yield (Sipes and Schmitt, 2000). At lower nematode populations, pineapple yield is limited by soil fertility and inherent soil physical

factors. *R. reniformis* becomes the major limiting factor at populations above 600 nematodes/250 cm³ soil (Sipes and Schmitt, 2000).

Disease complexes

Infection by *R. reniformis* increases the severity of pineapple mealybug wilt disease in the field. Co-infection of pineapple by the reniform nematode and the Pineapple mealybug wilt associated virus reduces fruit size and subsequent pineapple yield (Sipes *et al.*, 2002). Co-infection also resulted in increased early flowering by pineapple. The presence of the virus did not impact nematode reproduction, however (Sipes *et al.*, 2002).

Pratylenchus

The root lesion nematode *P. brachyurus* was described originally from pineapple roots in Hawaii (Godfrey, 1929). It is prevalent and of economic importance throughout the equatorial tropics in Brazil, Côte d'Ivoire, Hluhluwe in northern Natal (South Africa), Nigeria and Uganda (Guérout, 1975; Zem and Reinhardt, 1978; Bafokuzara, 1982; Keetch, 1982; Dinardo-Miranda *et al.*, 1996b; Daramola *et al.*, 2013a). *Pratylenchus* is also the major nematode problem in Indonesia and Malaysia (Lisnawita *et al.*, 2011). Although present, it is of limited importance in higher latitudes of the subtropics, such as the Caribbean, Hawaii, Australia or the Cape Province in South Africa (Guérout, 1975; Keetch, 1982; Rohrbach and Apt, 1986; G.R. Stirling, Australia, 2017, personal communication). Other lesion nematodes such as *Pratylenchus coffeae* and *Pratylenchus zae* have been observed in some pineapple production areas, but there is no information on their pathogenicity to pineapple.

Symptoms of damage

Black lesions caused by *P. brachyurus* develop in the roots at the point of nematode infection. The developing necrosis may extend progressively over the whole surface of the root as the nematodes feed and move through the root. Lesions are surrounded by dead and discoloured epidermal cells, and may extend throughout the parenchyma (Fig. 20.5) (Godfrey, 1929; Keetch, 1982). In the later stages of infection,



Fig. 20.5. Pineapple roots with lesions caused by infection of *Pratylenchus brachyurus*. (From Sipes *et al.*, 2005.)

the parenchyma is destroyed and the cortex separates from the central cylinder (Guérout, 1975). Secondary roots and root hairs are also destroyed by this nematode, leading to a root system composed of poorly developed primary roots. The damage to parenchyma tissue is not generally visible in the field as pineapple roots are rapidly and heavily suberized.

Infection by *P. brachyurus* decreases plant growth rate, delays leaf emergence and reduces leaf weights by 35–40% (Guérout, 1975; Lacoëuilhe and Guérout, 1976; Sarah, 1986). Leaves turn yellow and then red, lose turgidity, and their tips wither (Py *et al.*, 1984). Foliar symptoms result from deficient water and mineral supply to the pineapple plant and are especially noticeable if fertilizers are applied as granules to the soil before planting, as fertilizer absorption is suppressed by nematode damage. Foliar application of fertilizer decreases nematode influence on plant growth because leaves absorb nutrients and this compensates for decreased root function (Lacoëuilhe and Guérout, 1976).

Biology and life cycle

P. brachyurus is a migratory endoparasite. Males are rare, and reproduction is by mitotic parthenogenesis (Roman and Triantaphyllou, 1969). The life cycle of *P. brachyurus* may be completed within the roots. Thus, high populations of the nematode can develop quickly and cause the rapid destruction of the cortical parenchyma (Guérout, 1975).

Survival and dissemination

Under laboratory conditions, *P. brachyurus* survives from 20 to 22 months in fallow soil, as long as viable root fragments are present in the soil (Guérout, 1975). If root fragments are absent from the soil, survival without a host is limited to approximately 7 months. After 35 days at 44°C, only 25–50% of an original South African population survived (Keetch, 1977). In Côte d'Ivoire, *P. brachyurus* is sometimes disseminated when infected suckers are used as seed. The nematode possibly survives in the roots that wrap around the butt of the pineapple stump.

Environmental factors affecting parasitism

The optimum temperature for *P. brachyurus* development is 25–30°C (Olowe and Corbett, 1976). This temperature range encompasses the yearly average soil temperatures in many pineapple-growing areas. Although nematode movement is inhibited by soil temperatures above 40°C (Olowe and Corbett, 1976), many plantations are located on sandy soils, which are very favourable to *P. brachyurus* mobility.

With relatively constant soil temperatures, the root lesion nematode responds primarily to changes in soil moisture. If pineapple is planted during the dry season, nematodes in the roots will remain at low levels, increasing several weeks after the return of regular rainfall (Fig. 20.6a). When planted during the rainy season, nematode population densities in the roots increase rapidly after approximately 3 months (Fig. 20.6b). If soil moisture remains favourable, root populations remain relatively stable until forcing, and then decline. Approximately 20 mm rainfall/10 days is required in Côte d'Ivoire to maintain high root populations of *P. brachyurus* (Sarah and Hugon, 1991).

Root populations of *P. brachyurus* increase rapidly in acid soils and very slowly when pH exceeds 5–5.5 (Sarah *et al.*, 1991). In Côte d'Ivoire, *P. brachyurus* competitively displaces *Meloidogyne* spp., as the rapid destruction of root tissue by the root lesion nematode seems to prevent the establishment of the root knot nematode (Guérout, 1965).

Other hosts

The root lesion nematode has a wide host range that includes 100 recorded plant species, many

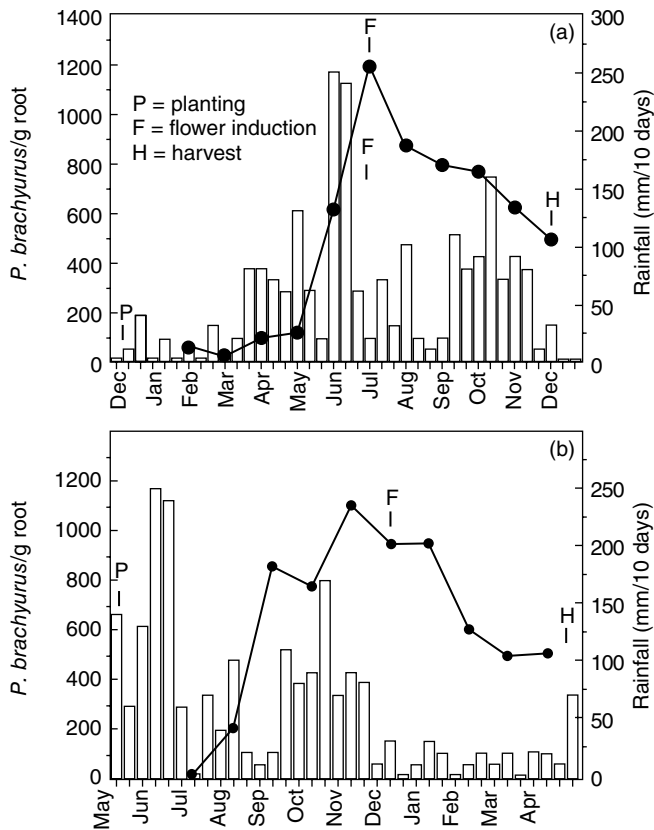


Fig. 20.6. Populations of *Pratylenchus brachyurus* in the roots of *Ananas comosus* cv. Smooth Cayenne related to rainfall in the Côte d'Ivoire. (a) Pineapple planted in December before the main dry season. (b) Pineapple planted in July at the end of the rainy season. (From Sipes *et al.*, 2005.)

of them grasses found in the natural savannahs where pineapple is cultivated (Luc and de Guiran, 1960). Maize and cassava are very good hosts for root lesion nematode, and these plants should not be used as rotation crops with pineapple.

Disease complexes

P. brachyurus may infect galls caused by *M. javanica* and cause the rapid breakdown of the gall and death of the root tip (Godfrey, 1929). Guérout (1975) demonstrated an interaction between *P. brachyurus* and pytheaceous oomycetes. The oomycete–nematode combination results in plant damage greater than that caused by the nematode alone. In Ghana, *Pratylenchus* has been implicated as one agent in red leaf disease of pineapple (Awuah, 2008).

Economic importance and damage threshold

In South Africa, inoculation with 200 *P. brachyurus* decreased plant growth by 25% after 10 months. This compares with a decrease of 10% caused by similar inoculation with *M. javanica* (Keetch, 1982). The damage caused by *P. brachyurus* can be severe, with yield losses reaching 30% for the plant crop and 80% for the first ratoon crop in Côte d'Ivoire (Lacoeuilhe and Guérout, 1976; Sarah, 1986). The damage threshold is partially determined by the planting date, because soil moisture and temperature influence nematode population growth rate and the capacity of the plant to tolerate infection. For example, dry conditions combined with *P. brachyurus* infection cause a drastic reduction in sucker development in Côte d'Ivoire

(Sarah, 1987). The linear relationship between initial population density of *P. brachyurus* and average fruit weight for pineapple planted just before the rainy season in Côte d'Ivoire (Fig. 20.7) indicates that the damage threshold is very low under these conditions (Sarah, 1986).

Management Measures

The primary emphasis of nematode management in pineapple is on protection of the young, growing root system. Reduction of nematode inoculum in the soil prior to planting or reduction of nematode population growth rate once plants are established in the field is the goal of management. Pre-plant control of nematodes is most important, as damage to the developing roots of the young plant results in poor plant growth (Godfrey, 1936). Pre-plant tactics to suppress nematode inoculum include the application of nematicides, rotations with non-host crops, fallowing and soil amendments. Post-plant management options are currently limited to nematicide application.

Nematicides

From the beginning of commercial pineapple production in the 1920s, the pineapple industry came to rely on a chemical-dependent cropping system. A century later, chemical nematicides

remain the primary means of managing plant parasitic nematodes in pineapple, regardless of the nematode species involved. Pre-plant or at-plant soil treatments protect the root system of the young pineapple plant against nematodes. Treatments can be applied as pre-plant fumigation, at-plant incorporation of granular nematicides, or pre-plant nematicide application via drip irrigation (Rohrbach and Apt, 1986; Apt and Caswell, 1988). An effective nematode management strategy is based on the crop cycle length and the number of ratoons desired. Protecting the root system for a minimum of 6 months is necessary, and 8–12 months of control is preferred, if ratoon crops are to be harvested.

Pre-plant fumigation treatments have changed over the years. At one time, products such as EDB, DBCP and methyl bromide were commonly used in the pineapple industry (Py *et al.*, 1984). Increased environmental concerns and changing regulations have seen these products removed from the market. Pre-plant fumigation now involves the application of 1,3-dichloropropene at 224–336 l/ha (Rohrbach and Apt, 1986; Schneider *et al.*, 1995; Rabie, 2017). Minimal application rates are achieved with the use of a single chisel and sealing the planting bed with a plastic mulch (Sipes *et al.*, 1993). Successful fumigation is usually sufficient to protect the plant crop, but not subsequent ratoon crops. In Côte d'Ivoire, non-fumigant nematicides are used as a pre-plant control.

Non-fumigant nematicides are typically applied as post-plant treatments, although their

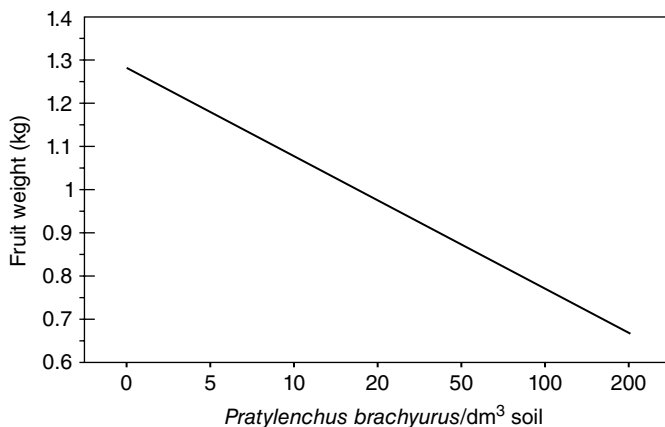


Fig. 20.7. Relationship between pineapple fruit weight and pre-plant soil population densities of *Pratylenchus brachyurus* in Côte d'Ivoire. (From Sipes *et al.*, 2005.)

usage is also undergoing changes. The range and types of non-fumigant nematicides have changed markedly in the past 30 years. Legislation designed to protect workers and the environment has drastically altered the nematicides that are registered for use in pineapple. Almost all carbamate and organophosphate nematicides, such as cadusafos, ethoprophos, fenamiphos, fosthiazate and terbufos, are not registered for use in the US or European markets. The systemic properties of some of the non-fumigant nematicides allow for foliar application during any point in the plant growth cycle. Generally, post-plant nematicide applications are only necessary if pre-plant fumigation is less successful and ratoon crops are planned. In many locations, post-plant applications are imperative for a successful plant crop. Recently, compounds such as DiTera, derived from the fungus *Myrothecium* sp., fluensulfone, fluopyram and spirotetramat have entered the market for nematode control and are being evaluated for use in managing nematodes in pineapple (Waisen and Sipes, 2014; B.S. Sipes, unpublished).

Post-plant application of non-fumigant nematicides requires good soil moisture conditions to promote movement in the soil and absorption by the plant, and to ensure that the nematode target is physiologically active (Sarah, 1980). The application of the nematicide with water through the drip irrigation system has the advantages of minimizing worker exposure and delivering the product directly to the site of action (Apt and Caswell, 1988; Sipes and Schmitt, 1995).

Research is also investigating the use of plant-induced and acquired systemic resistance as a nematode control tactic in pineapple. Laboratory and glasshouse tests suggest that applications of the systemic acquired resistance inducer acibenzolar-s-methyl may hold promise, because nematode reproduction is reduced by 50% with a single application (Chinnasri *et al.*, 2006a). Methyl jasmonate and salicylic acid application to the low acid hybrid MD-2 reduced populations of *R. reniformis* by 67 and 56%, respectively, in glasshouse studies (Soler *et al.*, 2013).

Cultural practices

Pineapple growers are often specialized in pineapple production. Consequently, the crop is

grown in long-term monoculture. Some fields in Côte d'Ivoire have been producing continuous pineapple for 30 years, while fields in Hawaii have produced pineapple for nearly a century.

Pineapple is essentially a perennial plant. After fruiting, the pineapple plant produces a slip that gives rise to the next fruit. This process of producing ratoon stumps can continue indefinitely. Fruit from second ratoons and later tend to be smaller in size than those from the plant crop. Commercial growers decide when to re-plant based on whether the ratoon fruit has become too small or too sparse. Therefore, pineapple crop cycles can be very long, for example 8 years in South Africa, or much shorter, for example 2.5 years in Hawaii. Fields typically are left fallow during the period between pineapple crops (called the intercycle). The duration of the intercycle is dictated by economics and pest control considerations. Solarization used during the intercycle in conjunction with cover crops and laying of mulch prior to planting has the potential to reduce the use of nematicides (Hooks *et al.*, 2010). The success of the intercycle in reducing nematode populations is also influenced by the type of fallow (e.g. clean versus 'natural' fallowing), soil moisture conditions and the host range of the nematode species involved.

Clean fallow

Weed-free fallow can be used to decrease nematode populations, although keeping a field free of weeds is difficult. An additional problem is that pineapple stumps can produce root tissue long after the shoots are destroyed, and volunteer pineapple can support nematode reproduction (Ko and Schmitt, 1993; B.S. Sipes and K.-H. Wang, unpublished). Weeds, such as nightshade and pigweed (*Amaranthus* spp.), growing during a 1-year fallow period supported high populations of root knot and reniform nematodes in Hawaii (W.J. Apt, unpublished). In Thailand, root knot nematodes were extracted from the rhizosphere of *Dactyloctenium aegyptium*, *Praxelis clematidea* and *Vernonia cinerea* weeds growing in pineapple fields (Hemniam *et al.*, 2016). In Côte d'Ivoire, *P. brachyurus* was found on 15 common weed species (Goly and Têhé, 1997). Even small weed seedlings have a root system capable of supporting significant numbers of

nematodes. Fields can be maintained nearly weed free by the application of herbicides, or through periodic cultivation. An added benefit of cultivation is that it brings deeper soil layers to the surface, exposing nematode eggs and juveniles to ultraviolet radiation and desiccation. In addition to soil erosion concerns, a possible problem with deep cultivation is that it brings the deeper weed seed bank to the surface, and may result in increased weed germination. As with all pest management strategies, multiple pests should be considered.

Although nematode populations decline during a clean fallow, it is virtually impossible to eradicate them. Even after fallow periods as long as 2 years, residual inoculum can remain (Godfrey, 1936; Guérout, 1975). Additionally, some nematode species have life history strategies that include cryptobiotic capacities, such as dauer stages that allow survival despite environmental extremes, or anhydrobiotic stages that can survive in a quiescent state. For example, *R. reniformis* juveniles can withstand severe dehydration under slow dehydration regimes (Womersley and Ching, 1989). The success of fallowing will depend, to a degree, on the nematode species involved.

The pineapple industry in Hawaii practices a 6- to 12-month clean fallow period between plant cycles. Fallow periods hasten the decline of reniform nematodes in soil, but moisture plays a role in determining the extent of population decline. *R. reniformis* can survive for as long as 1.5 years in desiccated, fallow soils (Apt, 1976; Tsai and Apt, 1979). In Côte d'Ivoire, 6 weeks fallow can reduce populations of *P. brachyurus* by half (Guérout, 1975).

Clean fallow can be a problem on large plantations, as it is energy intensive and may not be economically justifiable. In addition, erosion, one of the most important problems facing modern agriculture, may be increased by fallow. The absence of a cover crop may reduce soil fertility by slowing the addition of organic matter and decreasing the retention of soluble nutrients in the soil. Fallow may decrease the population densities of beneficial microorganisms, such as endomycorrhizae, as has been observed in Côte d'Ivoire (Sarah, 1987).

Crop rotation and cover crops

Because of some of the problems associated with clean fallow, planting cash crops in rotation or

non-host cover crops may be desirable. Both monocots and dicots have been evaluated for nematode control in pineapple.

Few cash crops are used in rotation with pineapple. Banana has been rotated with pineapple in Central America, Martinique and China (Ternisien and Ganry, 1990; Araya-Vargas and Calvo-Pineda, 2010; Wang *et al.*, 2015). In some of these cases, pineapple has been used to reduce pests associated with banana. In China, sugarcane rotated with pineapple led to significant improvements in soil ecology (Zheng *et al.*, 2003; Xi *et al.*, 2010). Both sugarcane- and banana-pineapple rotations are multiple-year cycles and involve quite different mechanization that may limit their use in pineapple. Consequently, intercycle cover crops or green manure crops have received more attention.

Numerous dicotyledonous plants have been evaluated as potential intercycle cover crops. French marigold, *Tagetes patula*, reduced *R. reniformis* population densities (Nakasono, 1973; Ko and Schmitt, 1993), whereas *Tagetes erecta* and *Tagetes polynema* increased populations of the reniform nematode (Wang *et al.*, 2001). Sunn hemp (*Crotalaria juncea*) has shown the most promise in Hawaii. Sunn hemp is a poor host for *R. reniformis*, has allelopathic effects towards the nematode, enhances antagonistic microorganisms in the soil and adds nitrogen to the soil (Caswell *et al.*, 1991; Wang *et al.*, 2001, 2002, 2003). Sunn hemp is also not a good host for *P. brachyurus*, whereas *Mucuna* sp. proved to be a good host and did not suppress nematode multiplication in pineapple (Rabie and Tustin, 2007). *Brassica napus* and other mustard family crops have been evaluated for their biofumigation potential; however, many mustard species are excellent hosts to root knot nematodes, such as *M. javanica* (Wang *et al.*, 2001, 2002). In Côte d'Ivoire, *Crotalaria usaramoensis*, *Stylosanthes gracilis* and *Flemingia congesta* reduced *P. brachyurus* populations after 18 months, increased the nitrogen content of the soil and increased the fruit weights of the subsequent pineapple crop by 25–30% (Guérout, 1969). Some cover crops have significant potential for use in the management of plant parasitic nematodes in pineapple.

Grasses have also been studied to assess their value as intercycle cover crops. Rhodes grass (*Chloris gayana*) reduced soil populations of *R. reniformis* as well as or better than clean fallow (Caswell *et al.*, 1991). *C. gayana* and *Desmodium*

unicatum have been successful as rotation crops to reduce populations of *Hoplolaiminae* and *Meloidogyne* spp. in the Cape Province (Keetch and Dalldorf, 1980). Pangola grass (*Digitaria decumbens*) has potential as a rotation crop for pineapple as it apparently stimulates eclosion of *M. incognita*, and toxins produced by the roots affect juvenile survival (Ayala *et al.*, 1967; Haroon and Smart, 1983a). Plantings of *D. decumbens* eliminate *M. incognita* after 1 year, and *Criconemella* spp. and *Helicotylenchus* spp. after 18 months. *D. decumbens* is a poor host for *M. javanica* (Haroon and Smart, 1983b), but *P. brachyurus* remained abundant even after 3 years of *D. decumbens* growth (Ayala *et al.*, 1967). Sugarcane is frequently grown in areas where pineapple is produced, and is generally considered a non-host for *R. reniformis*. Rotating pineapple with sugarcane may decrease some nematode problems, provided that weed hosts are not present. This strategy was attempted in Hawaii, with poor success. Sugarcane is a host for *P. brachyurus* in Hawaii, Brazil and Venezuela. When grown for 6 months, *Panicum maximum* increased pineapple yields better than did 6 months of *Chromolaena odorata* (Asteraceae), even though the latter showed a superior reduction of nematode populations. This last example demonstrates that the cover crop that gives the best nematode reduction will not necessarily result in the best yield of the subsequent pineapple crop.

Organic improvements and soil amendments

The addition of organic matter to pineapple soils is beneficial, as the decline of soil organic matter is faster in pineapple soils than under other crops (Py *et al.*, 1984). The addition of organic matter may have direct and indirect effects on plant parasitic nematodes. For example, adding cassava residues or extracts of neem (*Azadirachta indica*) leaves to soil reduces *P. brachyurus* populations by 75 and 72%, respectively, in Nigeria (Egunjobi and Larinde, 1975). Research on amendments with animal wastes has shown promise. Poultry manure added to pineapple trash reduces nematode damage and provides a means to handle animal wastes (Agu, 2008; Ch'ng *et al.*, 2013; Daramola *et al.*, 2013b). The addition of animal wastes probably has indirect effects on nematodes.

Linford (1937) found that adding organic matter to soil increased the activity of nematode-trapping fungi. In Hawaii, H.W. Klemmer and R. Nakano (unpublished) found that incorporating pineapple plant residues into the field (rather than burning them) significantly increased nematode antagonists in the soil, reducing reniform nematode populations. Much of the observed beneficial effect of organic matter incorporation is probably due to its stimulatory effect on the predators and parasites of nematodes. The challenge with organic amendments is the volume required, often in the tonnes/ha, and costs associated with transporting the amendments to the pineapple field.

Resistance and tolerance

Nearly all pineapple cultivars and clones support nematode reproduction. However, the level of reproduction and the tolerance to nematode infection vary widely. Tolerance to reniform and root knot nematodes is manifested in the pineapple cultivars and clones that root more vigorously. Long-term cultivation of Smooth Cayenne has resulted in an indirect selection for greater tolerance and resistance as compared with other less intensively grown cultivars in Hawaii (Sipes and Schmitt, 1994a).

Many pineapple cultivars and clones have been evaluated for resistance and tolerance to plant parasitic nematodes. Host-plant resistance has become a desired option as chemical controls have become limited and less socially acceptable (Soler *et al.*, 2009). Collins and Hagan (1932) assessed the tolerance of several pineapple clones to *M. javanica*. They found that Cayenne was very intolerant of nematode infection, whereas Wild Brazil and an F_1 hybrid from Wild Brazil $\times$ Cayenne were much more tolerant, if not immune to damage from nematode infection, as measured by shoot weight and root length (Collins and Hagan, 1932; Hagan and Collins, 1935). Sipes and Schmitt (1994a) found that the same cultivars supported reproduction of *M. javanica* but were tolerant to infection in that plant growth was not affected. *A. comosus* var. *ananasoides* and three other selections were reported as resistant to *M. incognita* in Puerto Rico (Ayala, 1961, 1968; Ayala *et al.*, 1969). Dinardo-Miranda *et al.* (1996a) found that among 13 cultivars evaluated, only Huitota supported significantly

lower populations of *M. incognita*. Four accessions from the Venezuelan Amazonian region were resistant to *M. incognita*, although two of them appeared intolerant to the nematode (Suárez and Rosales, 2008). *A. comosus* var. *ananasoides*, Venezuelan and two other clonal selections were resistant to Puerto Rican populations of *R. reniformis* (Ayala, 1961, 1968; Ayala *et al.*, 1969). In Hawaii, 18 cultivars were assessed for reniform nematode resistance, including two *A. comosus* var. *ananasoides* lines and two *A. comosus* var. *ananasoides* hybrids, and all supported reniform nematode reproduction (Sipes and Schmitt, 1994a). *A. comosus* var. *ananasoides* is an excellent host for *P. brachyurus* in Côte d'Ivoire (Py *et al.*, 1984). The Queen group of pineapple and *A. comosus* var. *bracteatus* are extremely susceptible to *P. brachyurus*. All of the 14 pineapple cultivars evaluated by Dinardo-Miranda *et al.* (1996b) were good hosts to *P. brachyurus*, as well as the 21 pineapple cultivars evaluated by Sarah *et al.* (1997). However, cultivars from the Pérola group appeared slightly (although not significantly) less infected in several experiments of the latter study.

Genetic engineering of pineapple to incorporate resistance to nematodes has been undertaken (Wang *et al.*, 2009). Early efforts attempted to introduce protease inhibitors as a method to control nematode development (Sipes, 2010). Transgene expression levels, however, were probably not sufficiently high to affect nematode reproduction in these plants. Nematode infection of pineapple causes changes in gene expression, and in time we may be able to exploit these changes for the control and management of nematodes (Chinnasri *et al.*, 2006b, 2016; Moyle and Botella, 2014).

Biological management

Linford, a researcher at the Pineapple Research Institute, Oahu, Hawaii, was a pioneer in the biological control of nematodes. Many nematode-parasitizing fungi have been identified in pineapple soils, including *Arthrobotrys oligospora*, *Catenaria anguillulae*, *Harposporium anguillulae* and *Stylopaga hadra* (Linford, 1937). In laboratory and greenhouse experiments, Linford (1937) and Wang *et al.* (2003) examined the potential

of incorporating organic matter to stimulate the activity of nematode predators and parasites in the soil. The incorporation of organic matter resulted in increased populations of free-living nematodes that are prey for nematode-parasitizing fungi, resulting in increased fungal populations (Linford, 1937). The addition of chopped pineapple material to soil at a rate of 37–111 kg/m³ soil significantly reduced galling caused by root knot nematode, as determined by bioassay (Linford, 1937; Linford *et al.*, 1938). Wang *et al.* (2003) found that sunn hemp amendment increased the number of nematode-trapping fungi in the soil. Linford also investigated the potential for using several fungi as biological control agents (Linford and Yap, 1939). In small pot tests, they observed that the addition of *Dactylella ellipsospora* reduced plant injury caused by the root knot nematode, although the results were confounded by the presence of other natural enemies of nematodes in the treatment.

Potential biological control agents must be tested in field soil. Selected strains of *Purpureocillium lilacinum* are available commercially. In Kenya, local populations of *Purpureocillium lilacinum* and *Trichoderma* spp. reduced *M. javanica* reproduction, demonstrating their potential in pineapple (Kiriga, 2017). *Pasteuria* spp. have been detected on root knot, reniform and lesion nematodes in pineapple and other cropping systems (T.E. Hewlett, K.H. Wang and B.S. Sipes unpublished). The activity of these introduced biological control agents may differ and will depend on the biotic and abiotic characteristics of the soil to which they are applied (Linford and Yap, 1939). Although biological control is a potential component of a nematode management programme in pineapple, it does not currently play a major role in nematode management in any of the world's commercial pineapple cultivation.

Methods of Diagnosis

Sampling

Before planting, soil samples should be taken to a depth of approximately 30–40 cm with a trowel or soil-sampling tube. Ideally, the soil should be in a condition of good tilth suitable for

sampling. A composite soil sample consisting of 30 cores/100 m² is adequate for most analyses. If the nematode population estimates are required to a certain level of accuracy, then pre-treatment samples taken on a quadrant basis can be used to estimate the numbers of samples required for a given degree of accuracy (Barker *et al.*, 1986).

Samples taken during crop growth should be removed from between plants within the plant row and in the root zone. A composite sample consisting of 10 cores per 15–20 m of row provides an accurate estimate of the nematodes present. Samples should be bagged and protected from temperature extremes and desiccation until processed. For research studies or IPM monitoring, samples should be taken on a monthly basis, commencing about 2 months post-plant. Rotating the location where the soil sample is collected in a row will allow plants to recover between sampling times and avoid negative effects on plant growth related to sampling.

Nematode extraction

The nematode extraction technique used depends on the objectives of the sampling programme, the nematode species present in the soil or the roots and the stage in the crop cycle. Soil-dwelling root knot nematode juveniles and adult stages of the reniform nematode can be recovered by processing soil with Baermann funnels, by a combination of Cobb sieving and centrifugation–flotation, or by processing root samples using mist apparatus. Females and associated egg masses can be visualized by staining root segments. Eggs of root knot and reniform nematodes can be collected from the roots using an NaOCl solution (Barker *et al.*, 1986). For the endoparasitic root lesion nematode, density estimates are obtained by extracting nematodes from soil and roots using centrifugal–flotation or Baermann funnels (Coolen and d’Herde, 1972; Hendricks *et al.*, 1976). Roots can also be macerated or digested enzymatically to release nematodes for counting (Alvarado-Soto and Lopez-Chaves, 1981; Barker *et al.*, 1986).

The inoculum of root lesion nematode prior to planting is sometimes estimated in Côte d’Ivoire by using a maize bioassay. The bioassay is

especially helpful if initial population densities are low, and the bioassay is performed by placing a soil sample into several pots and sowing maize in the pots. The root lesion nematodes are extracted from the soil and roots after 5 weeks, to allow nematode reproduction, increasing the probability of detecting the nematode.

Determination of populations and crop loss

Studies on nematode damage to pineapple must be undertaken in sufficiently large plots comprising 80–120 plants. Each treatment should have a minimum of four replications, with five used in general practice. Each experiment should include appropriate controls: a non-treated control, a standard treatment control (plantation practice) and an irrigated control (if the experiment is irrigated) (Apt and Caswell, 1988).

Observations on plant growth are typically non-destructive, using D-leaf measurements and estimated plant weights. Plants are sometimes uprooted for inspection, or soil profile samples are taken to assess root development and nematode distributions within the soil profile. Soil samples for nematode assessment are taken at random from those beds designated ‘non-yield’. Nematode soil samples are taken from the inside edge of these beds in the treated area, while the centre beds are reserved for yield assessment and are not sampled to prevent root system damage. Soil samples can be collected on a rotation basis, so that each soil core is removed from the soil around plants that have not been sampled previously.

At harvest, fruit are picked, size classed and the fruit and crown weights determined per size class. Individual fruit weights rather than composite weight of all fruit from a plot provides more statistically robust data. Fruit can be analysed for sugar and acidity, the number of fruit-lets and deformity. The specifics of the analysis are determined by the objectives of the research.

Summary of Nematodes in Pineapple

Plant parasitic nematodes can be devastating to pineapple, reducing total yields and altering fruit

size distributions. Nematode control methods have changed dramatically in the past 25 years, and are likely to change even more in the next decade. Many effective nematicides, including several soil fumigants, carbamates and organophosphate nematicides, have been removed from the market, and new nematicides have yet to be evaluated in the pineapple system. Consequently, alternatives to nematicides are more important than ever. Manipulation of the fallow period with intercycle cover crops and maintenance of soil moisture hold promise for increasing nematode control. Living mulches and nematode antagonists may eventually augment or replace traditional

chemical nematicides. Plant resistance and biological (biological agents) control are promising, although challenging, approaches for the coming years. Crop management from seed selection to fertilizer application to irrigation play an important role in nematode management by improving the plant's tolerance of nematode damage. However, the most effective means of controlling plant parasitic nematodes on pineapple in intensive production systems remains chemical nematicides. The use of fumigant nematicides and non-fumigant nematicides, when available, provides very effective nematode management.

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21 Nematode Parasites of Cotton and other Tropical Fibre Crops*

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Several fibre crops are important agricultural commodities in the subtropics and tropics, with cotton having the greatest total production (estimated at 26 Mt for 2014). Although there are four cultivated species of cotton, upland cotton *Gossypium hirsutum* accounts for approximately 90% of the world's production. Other important fibre crops include jute (*Corchorus capsularis* and *Corchorus olitorius*), kenaf (*Hibiscus cannabinus*) and roselle (*Hibiscus sabdariffa*). World production of jute was approximately 3.5 Mt in 2013 and kenaf was 0.3 Mt in 2008. Although multiple nematode species are associated with each of these fibre crops, the root knot nematodes are responsible for the greatest proportion of all reported yield losses due to nematodes.

Cotton

Upland cotton is a relatively drought-tolerant crop by virtue of its long taproot, which may reach depths of greater than 1 m. The importance of the taproot to cotton growth may be a factor in cotton being generally intolerant of the damage caused by parasitic nematodes, especially in climates where soil moisture for crop growth is often limited. Because cotton is grown

as a cash crop, it is frequently grown in a monoculture system that favours the development of a nematode community dominated by one or a few parasitic species (Starr *et al.*, 1993). Monoculture of cotton occurs in both large-scale production systems and in resource-poor production systems in marginal areas of countries of Africa and elsewhere. This chapter focuses on nematode species known to suppress yield of *G. hirsutum*. For other reviews of nematodes parasitic on cotton, the reader is referred to Bridge (1992) and Starr (1998).

Meloidogyne

Distribution

Of the 98 or more described *Meloidogyne* species, only three are known to be pathogenic to cotton, *Meloidogyne acrona*, *Meloidogyne incognita* (host races 3 and 4) and a few populations of *Meloidogyne enterolobii*. Early literature refers to the subspecies *M. incognita acrita*, but this is no longer recognized as a valid taxon and all such reports are now considered to refer to *M. incognita*. Of all of the root knot nematodes, *M. incognita* has the greatest frequency distribution in warm temperate to tropical agroecosystems, accounting for

*A revision of the chapter by J.L. Starr, R.G. Carneiro and O. Ruano in the second edition.

more than 60% of the identified infestations (Sasser and Carter, 1985). Thus, this nematode has been reported from nearly all cotton production regions, especially where soils are coarsely textured (Robinson *et al.*, 1987; Starr *et al.*, 1993). In some cotton production regions with conducive soils, *M. incognita* is present in more than 50% of the cotton fields in the USA (Starr, 1998) and an estimated 28% of fields in Brazil (Asmus, 2004). In regions of the USA and Brazil where cotton is grown on finely textured soils with higher contents of clay, *M. incognita* is rarely detected. Unfortunately, few other regions have been surveyed in sufficient detail to permit such estimates of frequency distribution. *M. incognita* host race 3 is the most common host race found on cotton. In contrast to *M. incognita*, *M. acronea* is known only from the Shire valley in Malawi and other semi-arid regions of southern Africa (Page, 1983). *M. acronea* may be indigenous to this region, which is also a habitat for the wild precursor of some cottons, *Gossypium herbaceum* var. *africanum*.

Symptoms

As with many nematode-incited plant diseases, accurate diagnosis based on foliar symptoms is difficult. The general symptoms of disease include stunting, chlorosis, incipient wilting and a general unthrifty appearance (Fig. 21.1). Silva *et al.* (1997) reported that a common foliar

symptom induced by *M. incognita* was a 'speckled' appearance of the interveinal tissues of the leaves (Fig. 21.2). Root galling of cotton by *M. incognita* is often indistinct, especially early in a cropping season and with low to moderate levels of infection. Under these conditions, the galls are less than twice the diameter of non-infected roots and are easiest to detect on lateral roots. As the crop nears maturity and the nematode population densities increase, there is an increased frequency of more heavily galled roots and an increase in the size of the galls (Fig. 21.3). The root symptoms induced by *M. acronea* are distinct from those of *M. incognita*. Mature females of *M. acronea* are often exposed on the root surface, with very limited galling. Root elongation often ceases following infection by *M. acronea*, but there may be a proliferation of lateral roots from the infection site (Fig. 21.4). Roots of cotton infected by *M. acronea* are said to have a 'turned-aside' appearance, which is due to cessation of growth of the taproot accompanied by increased formation of lateral roots (Page, 1983). Galls on cotton caused by *M. enterolobii* are larger than those caused by *M. incognita* (Ye *et al.*, 2013).

When examining cotton roots for symptoms of infection by root knot nematodes, it is important that plants be removed from the soil carefully so as to recover a high proportion of the lateral roots, where root galls are most evident. Pulling plants from the soil to estimate root



Fig. 21.1. Healthy cotton (left) compared with cotton stunted due to *Meloidogyne incognita*. (Photograph courtesy of R.F. Davis.)

galling will result in the loss of most of the weak symptomatic lateral roots.

Biology

The biology of *M. incognita*, *M. acronea* and *M. enterolobii* is similar to that of other *Meloidogyne* species. *M. acronea* reproduces almost entirely by amphimixis, whereas *M. incognita* and *M. enterolobii* are strictly parthenogenetic. *M. incognita* is favoured by warmer soil temperatures (optimum is $\sim 28^{\circ}\text{C}$) and does not survive long periods of freezing temperatures. No data are available on optimal temperatures for *M. acronea*, but with its known distribution it is likely to behave similarly



Fig. 21.2. Speckled leaf symptom of cotton caused by *Meloidogyne incognita*. (Photograph courtesy of R. Galbieri.)

with respect to the effects of temperature on development and survival. Detailed information on the biology of *M. incognita* can be found in previous reviews (Starr, 1998; Moens *et al.*, 2009).

Population dynamics

In conducive soils with favourable temperatures and adequate moisture, the host status of the crop will govern nematode population densities. Except for a few resistant *G. hirsutum* genotypes, cotton is a susceptible host that can support population densities of over 10^4 eggs and juveniles/500 cm^3 soil (Veech and Starr, 1986). Population densities may increase several hundred-fold during a cropping season, especially when initial densities are very low. Nematode reproduction factors (R_f = final population/initial population) at crop maturity are related inversely to the initial population densities (Veech and Starr, 1986; Starr *et al.*, 1989). The predominant developmental stage of *M. incognita* during the growing season is eggs (Barker *et al.*, 1987). Soil texture can affect *M. incognita* population levels significantly (Mueller *et al.*, 2010). Reproduction of *M. incognita* is greatest in sandy soils and decreases as the percentage of silt and clay increases (Koenning *et al.*, 1996; Monfort *et al.*, 2007). The presence of other nematode species parasitic on cotton can affect the population dynamics of *Meloidogyne* spp. Gay and Bird (1973) reported that *Pratylenchus brachyurus*

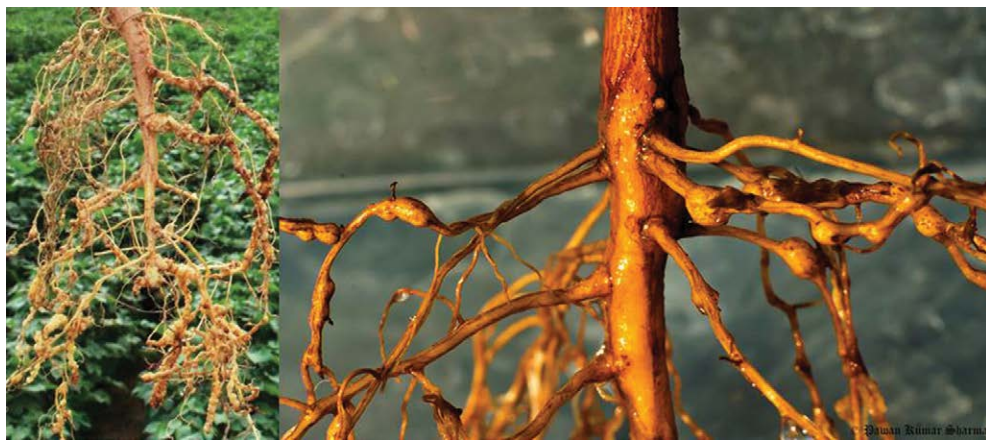


Fig. 21.3. Severe root galling of cotton caused by *Meloidogyne incognita*. (Photographs courtesy of R. Galbieri (left) and P. Kumar (right).)

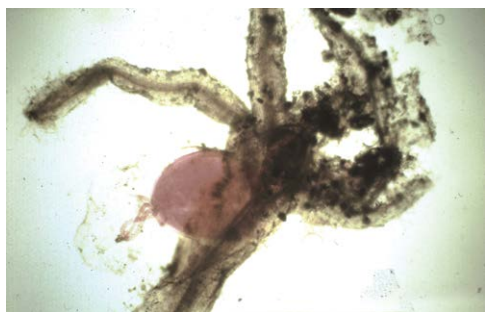


Fig. 21.4. *Meloidogyne acronea* female and proliferation of lateral roots from the feeding site. (Photograph courtesy of J. Bridge.)

suppressed population development of *M. incognita*. Bird *et al.* (1974) and Kraus-Schmidt and Lewis (1981) reported that *M. incognita* could not compete with *Hoplolaimus columbus* and that *H. columbus* would replace *M. incognita* as the dominant species in fields infested with both nematodes. Diez *et al.* (2003) documented antagonistic effects when *M. incognita* and *Rotylenchulus reniformis* concomitantly infected cotton, with *M. incognita* more susceptible to antagonism from *R. reniformis* than the reverse. Aryal *et al.* (2011a,b) showed that *M. incognita* and *R. reniformis* could induce systemic acquired resistance against each other, which likely contributed to the observed antagonism. Some soil microbial communities can be naturally suppressive to nematodes (Timper *et al.*, 2012; Adam *et al.*, 2014). *M. incognita* can also be suppressed by fungal pathogens that infect cotton and increase the rates of plant mortality (Starr *et al.*, 1989).

Survival

Most studies of survival of *Meloidogyne* species have focused on winter survival. Percentage winter survival is related inversely to autumn (fall) population densities (Ferris, 1985; Starr and Jeger, 1985) and may be related to reduced partitioning of nutrients from the host into the developing eggs at very high nematode population densities (Starr, 1988). Egg densities decline exponentially after crop harvest during winter months, due to the combined effects of hatch and mortality (Starr and Jeger, 1985). Population densities of infective juveniles (J2s) increase initially during the early winter months before declining in the late winter and early spring. The

survival of eggs within the egg mass is enhanced at temperatures less than 20°C in dry soils with low matric potential (–4 bars) due to the inhibition of hatch (Starr, 1993). *M. acronea* also survives the 6- to 7-month dry season as unhatched eggs within the egg mass or within the body of the dead female that has developed a thickened cuticle. Eggs in these structures are dormant and viable unless the soil becomes very dry (relative humidity of <97%). The limited (known) distribution of *M. acronea*, restricted to the Shire Valley in Malawi, may be related to the alluvial soils of the valley, which have greater moisture-holding capacity than other soils of the region (Page, 1984).

Damage thresholds

Susceptible cotton cultivars are highly intolerant of *M. incognita*, with damage thresholds in the range of 1–9 individuals/500 cm³ soil (Roberts *et al.*, 1985; Starr *et al.*, 1989). Cotton cultivars with resistance to *M. incognita* support lower levels of nematode reproduction and generally should have increased tolerance (Davis and May, 2003). Zhou and Starr (2003) reported that moderately resistant cultivars did not affect the damage threshold density; however, the resistant cultivars were more tolerant than the susceptible cultivars. Koenning *et al.* (2001) and Colyer *et al.* (1997) reported that some cultivars with moderate resistance to *M. incognita* responded to nematicide treatment of infested soil with a significant yield increase. The absolute and percentage losses to *M. incognita* in cotton increase as yield potential increases (Davis and May, 2005). Koenning *et al.* (1996) reported only minor influences of soil type on the damage functions of *M. incognita* on cotton in microplots. Monfort *et al.* (2007) and Overstreet *et al.* (2014) concluded that damage to cotton from *M. incognita* was tied closely to soil texture, with less damage in clay soil or where sand overlaid a clay soil.

Disease complexes

M. incognita on cotton is known to be involved in numerous disease complexes (synergistic interactions), especially with the vascular wilt pathogen *Fusarium oxysporum* f.sp. *vasinfectum* and with several other fungi causing seedling disease (Fig. 21.5). Roberts *et al.* (1985) reported that



Fig. 21.5. Symptoms of Fusarium wilt–root knot nematode complex of cotton. (Photograph courtesy of M.B. da Silva.)

the slope of the damage function for *M. incognita* on cotton was more negative in the presence of *E. o. vasinfectum* than in the absence of the wilt pathogen. Starr *et al.* (1989) reported that the interaction between the two pathogens was most evident when the nematode density exceeded the damage threshold and with intermediate levels of the wilt pathogen. Further, they reported that increased plant mortality early in the growing season was a major consequence of the disease complex. Resistance to Fusarium wilt can be broken by *M. incognita*, and wilt symptoms are more severe, develop more rapidly and with greater frequency when plants are also infected by *M. incognita* (Ruano *et al.*, 1984). However, race 4 of *E. o. vasinfectum* causes severe disease in the absence of *M. incognita* (Kim *et al.*, 2005) and may not be involved in a synergistic interaction. Interactions with seedling pathogens, such as *Rhizoctonia solani* and several *Fusarium* and *Pythium* species are well known (Brodie and Cooper, 1964). Interactions with *Thielaviopsis basicola* have also been documented (Walker *et al.*, 1998). In all of these interactions, there is greater incidence of the

wilt or seedling disease, with greater yield suppression, when cotton is infected with multiple pathogens than when only a single pathogen is present. Few interactions with other pathogens have been reported for *M. acronea*. It has been observed that the cyst-like appearance of mature females of *M. acronea* (Bridge *et al.*, 1976) may be due to the effects of oxidases secreted by *T. basicola*, which cause a tanning reaction of the nematode's cuticle (Page, 1983).

Management measures

CHEMICAL: the management of root knot nematodes in cotton has relied heavily on the use of nematicides. Numerous studies have demonstrated profitable increases in yield in response to nematicide applications. The fumigant 1,3-dichloropropene often provides a greater yield increase and greater suppression of final nematode densities than does the carbamate non-fumigant aldicarb (Kinlock and Rich, 1998). Seed treated with abamectin or thiodicarb may provide sufficient protection at lower nematode population densities (Monfort *et al.*, 2006;

Wheeler *et al.*, 2013). Fluopyram is a fungicide with nematostatic properties that can reduce nematode infection of plants (Faske and Hurd, 2015). Oxamyl may be applied after plant emergence and is typically used in conjunction with another nematicide applied at planting (Lawrence and McLean, 2002).

Instead of applying a uniform rate of nematicide to an entire field, the amount of nematicide applied can sometimes be reduced without allowing increased damage through the use of variable rates based on initial nematode densities and site-specific treatments (Mueller *et al.*, 2010; Overstreet *et al.*, 2014). Intensive sampling to estimate nematode densities is cost prohibitive, but soil texture measured by electrical conductivity along with other variables can be used to delineate nematode management zones, such that the variation in soil texture and edaphic factors is minimized within a zone and maximized among zones. Nematode population densities are typically correlated with those factors; therefore, densities can be estimated adequately by representative sampling of the zones. Management zones may be less useful in fields with little variability in soil texture. Fields must be sampled annually to estimate population densities of *M. incognita* in cotton reliably (Wheeler *et al.*, 2000).

CROP ROTATION AND SOIL AMENDMENTS: despite the extensive host range of *M. incognita*, several crop rotation systems that suppress nematode population densities and increase crop yields are known. These include rotations of cotton with groundnut (peanut; *Arachis hypogaea*) (Kirkpatrick and Sasser, 1984) and root knot-resistant cowpea (*Vigna unguiculata*) (Duncan and Ferris, 1984). Although some variability in the reproduction of *M. incognita* exists among maize (*Zea mays*) and sorghum (*Sorghum bicolor*) genotypes, resistant maize hybrids are not available and resistance in sorghum cultivars is poorly characterized. Pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), maize, groundnut, guar bean (*Cyamopsis tetragonoloba*) and leucaena bean (*Leucaena glauca*) are poor hosts for *M. acronea* and can be used to suppress nematode densities when grown in rotation with cotton (Page, 1983). Sorghum is a host

for *M. acronea* and not a suitable rotation crop. In crop rotation systems for the management of root knot nematodes, the beneficial effects may be greater if the non-host crop is grown for at least two seasons before planting susceptible cotton. However, when moderately resistant cotton was grown in rotation with susceptible cotton, most of the nematode-suppressive effects were established after a single season, although population densities rebounded quickly, even after three seasons of resistant cotton (Davis and Kemeraït, 2009).

Soil amendments with castor bean (*Ricinus communis*) cakes (Lordello and Sabino, 1985) and with various green manure crops, including some *Tagetes* spp., several *Crotalaria* spp. and velvet beans (*Mucuna pruriens*) (dos Santos and Ruano, 1987), will suppress *Meloidogyne* spp. and other nematodes. Such treatments might be particularly useful for resource-poor cotton farmers.

RESISTANCE: although most cotton cultivars are susceptible and intolerant of *M. incognita*, numerous sources of resistance have been summarized in previous reviews (Robinson, 2008; Davis and Stetina, 2016). DNA markers for resistance quantitative trait loci (QTLs) were identified on chromosomes 11 and 14 (Gutiérrez *et al.*, 2010; He *et al.*, 2014), which facilitated the creation of resistant cultivars. However, specific resistance genes have not yet been identified. In the cotton production areas of the western USA, cv. Acala NemX has been shown to have a competitive yield potential in infested fields and to suppress nematodes (Ogallo *et al.*, 1997). Additionally, the use of cotton with resistance to *M. incognita* will reduce yield losses in nematode-susceptible crops grown in rotation with resistant cotton (Ogallo *et al.*, 1999). In Brazil, several cotton cultivars with resistance to *M. incognita* and to multiple pathogens have been released (Cia *et al.*, 2001).

The *M. incognita*-resistant genotype Auburn 623 was reported to be susceptible to *M. acronea* (Page and Bridge, 1994), as were accessions of *Gossypium arboreum*, *G. herbaceum* var. *africanum* and *Gossypium barbadense*. One accession of *G. hirsutum* (UK 64) was found to have a moderate level of resistance to *M. acronea* (Page and Bridge, 1994).

Rotylenchulus

Distribution and symptoms

Two species of reniform nematodes, *Rotylenchulus parvus* and *R. reniformis*, are confirmed parasites of cotton, but few reports are available concerning the interaction of *R. parvus* and cotton. *R. reniformis* is noted especially for being associated with soils with higher silt and clay contents than soils in which root knot nematodes are commonly found (Robinson *et al.*, 1987; Starr *et al.*, 1993). *R. reniformis* is widespread in the cotton production regions of the southern USA (McLean and Lawrence, 2000) and southern Mato Grosso State in Brazil (Galbieri *et al.*, 2016), and appears to be replacing *M. incognita* as the dominant species in many fields. One unique trait of the reniform nematode is its spatial distribution in infested fields. Tihohod *et al.* (1992) reported that *R. reniformis* had a more uniform distribution in cotton fields than other nematode species. Robinson *et al.* (2000) and Westphal and Smart (2003) reported that *R. reniformis* was often found relatively deep in the soil profile, in some cases with more than 50% of the population occurring at depths greater than 30 cm.

The symptoms caused by *R. reniformis* are rather nondescript, as is typical for most parasitic nematodes, and may include stunted growth, poorly developed roots and chlorosis. Heavily infected roots may have a 'dirty' appearance, even after rinsing with water, due to the adhesion of soil particles to the egg masses (Fig. 21.6). Ferraz and Monteiro (1995) reported that whereas 'speckled' symptoms might occur in the interveinal tissue, the most visible effect was reduced growth. Due to the relatively uniform distribution of *R. reniformis* in many infested fields, symptomatic plants may not occur in distinct clusters. Rather, infected and stunted plants may be distributed so uniformly as not to be readily apparent.

Population dynamics and damage thresholds

In general, population densities of *R. reniformis* are at a minimum in the late spring and during the first month of a cropping season, and at a maximum as the crop nears maturity. Densities as high as 49,000 individuals/100 g soil have been detected (Jones *et al.*, 1959). *R. reniformis* is noted for its ability to survive periods of drought



Fig. 21.6. Egg masses (stained red) of *Rotylenchulus reniformis* on cotton roots and swollen *R. reniformis* female stained red (inset). (Photographs courtesy of S.R. Stetina.)

in an anhydrobiotic state (Tsai and Apt, 1979). The distribution of *R. reniformis* in soil below plough depths (>45 cm) apparently also improves survival. Robinson *et al.* (2005) reported that 20% of *R. reniformis* present in soil below 60 cm in the autumn survived the winter.

A damage threshold of 16 individuals/200 cm³ soil was reported from small pot tests (Sud *et al.*, 1984). Several studies have reported significant increases in cotton yield in response to nematicide application when initial densities of *R. reniformis* were in the range of 100–250 nematodes/100 cm³ soil. Koenning *et al.* (1996) reported that the relationship between initial densities and seed cotton yield fitted a linear model in several soil types, generally with more than 100 individuals/500 cm³ required to suppress yields by 10%.

Disease complexes

The reniform nematode forms disease complexes with *F. oxysporum* f.sp. *vasinfectum* (Prasad and Padeganur, 1980), *Verticillium dahlia* and with several seedling disease pathogens (Brodie and Cooper, 1964). Sankaralingam and McGawley (1994) reported that the combination of *R. reniformis* and *R. solani* did not affect the severity of seedling disease, but did result in lower overall growth and an increase in *R. reniformis* densities.

Management measures

The management of *R. reniformis* on cotton has relied primarily on nematicides and crop rotation. Numerous studies have reported higher yields of cotton following the application of a variety of nematicides, including foliar applications of oxamyl (Lawrence and McLean, 2000) and seed treatment with abamectin (Faske and Starr, 2007). Robinson *et al.* (2002) reported that, due to the depth of distribution of *R. reniformis* in some soils, yield response to fumigation could be improved by deeper placement of 1,3-dichloropropene. The repeated use of aldicarb has limited its effectiveness in some fields, due to the development of a microflora population that degrades the material rapidly (McLean and Lawrence, 2003). The use of geostatistics and management zones may improve the cost-effectiveness of reniform nematode management by better

targeting nematicide application (Davis *et al.*, 2013; Moore and Lawrence, 2013).

Several crops can be grown in rotation with cotton to suppress *R. reniformis* densities and to improve cotton yields, including maize (Westphal and Smart, 2003), reniform-resistant soybean (*Glycine max*) (Davis *et al.*, 2003), sorghum (Westphal and Smart, 2003), maize intercropped with black velvet bean (Curi, 1980) and wheat (*Triticum aestivum*) (Birchfield, 1983), and ruzizi grass (Congo grass, *Brachiaria ruziziensis*) in integrated crop–livestock systems (Asmus and Richetti, 2010).

No upland cotton cultivars with useful levels of resistance to *R. reniformis* are known. However, high levels of resistance, or even immunity, can be found in other *Gossypium* species (Davis and Stetina, 2016). The single dominant gene *Ren^{lon}* from the diploid species *Gossypium longicalyx* was introgressed successfully into upland cotton through a hexaploid bridging line to create two *G. hirsutum* germplasm lines, LOREN-1 and LOREN-2, both highly resistant to *R. reniformis* (Bell *et al.*, 2014). However, these lines were severely stunted, with limited growth and low yields in heavily infested soils. Several accessions of the tetraploid *G. barbadense* have moderate levels of resistance to *R. reniformis* (Yik and Birchfield, 1984). The *G. barbadensis* line GB713 is highly resistant to *R. reniformis* (Robinson *et al.*, 2004). GB713 has three QTLs associated with resistance to *R. reniformis* (Gutiérrez *et al.*, 2011). Recently, the germplasm line BARBREN-713 was registered as resistant to both *R. reniformis* and *M. incognita* (Bell *et al.*, 2015). Crosses between the *G. barbadense* line Tx110 and the root knot-resistant *G. hirsutum* M315 resulted in progeny resistant to both nematodes (Silvey *et al.*, 2003) but lacking the necessary yield potential for commercial release. Cotton accessions with tolerance to parasitism by *R. reniformis* have been identified (Cook *et al.*, 1997; Koenning *et al.*, 2000) and may be useful for limiting yield losses.

Pratylenchus

P. brachyurus has been associated with damage to cotton in southern USA and in Brazil. In the

USA, reports of pathogenicity on cotton were primarily from the states of Alabama and Georgia and were prior to 1980 (Bird *et al.*, 1971; Hussey and Roncadori, 1978). Starr and Mathieson (1985) were unable to confirm these reports working with a *P. brachyurus* population from Texas. In Brazil, *P. brachyurus* is the plant parasitic nematode most frequently associated with cotton, with up to 90% incidence in cotton fields in the western central region (Galbieri *et al.*, 2016). Lordello and Arruda (1957) reported *P. brachyurus* associated with stunted plants with small stems and poorly developed root systems. Carneiro *et al.* (1990) found that 45% of the cotton fields with sandy soils in Paraná were infested with *P. brachyurus*. Pathogenicity of *P. brachyurus* on cotton was demonstrated under greenhouse conditions with high initial population densities (Inomoto *et al.*, 2001; Machado *et al.*, 2012), although no correlation between population density and cotton yield or damage could be established under field conditions (Machado *et al.*, 2006; Galbieri *et al.*, 2016), which indicated that cotton was tolerant to *P. brachyurus* (Machado *et al.*, 2012).

P. brachyurus reproduces well on several grain crops, thus most cultivars of maize, sorghum and wheat are not likely to be good rotation crops for suppression of this nematode. *Crotalaria spectabilis*, some millet cultivars and black oat (*Avena strigosa*) are poor hosts for *P. brachyurus* (Inomoto and Asmus, 2010). Variation in the tolerance of cotton cultivars to *P. brachyurus* has been reported (Goulart *et al.*, 1997), but resistance is not available.

Pratylenchus sudanensis was reported as a pathogen of *G. barbadense* but not *G. hirsutum* in Sudan (Yassin, 1974), with potential yield reductions of 56–88% (Yassin, 1980). *P. sudanensis* can be involved in a Fusarium wilt disease complex (Saadabi and Yassin, 2007).

Hoplolaimus

Several *Hoplolaimus* species are pathogenic on cotton. *Hoplolaimus aegypti* is reported from Egypt, *H. columbus* from the USA and Egypt, *Hoplolaimus indicus* from India, *Hoplolaimus seinhorsti* from several countries in Africa and *Hoplolaimus magnistylus* from the USA (Donald *et al.*, 2013). All species apparently exhibit both

ecto- and endoparasitic associations with the host. A damage threshold for 10% yield loss has been reported at 70 *H. columbus*/100 cm³ soil (Noe, 1993). Root densities at 42 days after planting ranging from 200 to 600 nematodes/g root caused yield losses of 2–12% (Mueller and Sullivan, 1988). Significantly delayed crop maturity and 25% yield loss were observed when the population density at planting was 100 nematodes/100 cm³ soil (Bond and Mueller, 2007). *H. columbus* spatial distribution is influenced by soil texture, with greater densities correlated with high sand content (Holguin *et al.*, 2015). No resistance has been reported in cotton to *Hoplolaimus* species, although tolerance has been identified in some cultivars (Koenning and Bowman, 2005). Cultural practices, such as root destruction, immediately after harvest and winter cover crops (Davis *et al.*, 2000) or alteration of the planting date (Koenning *et al.*, 2003) were not effective in increasing cotton yields in fields infested with *H. columbus*.

Ectoparasitic nematodes

A few species of ectoparasites are pathogenic on cotton, but most of these are limited in distribution and, although of great importance where they occur, are of lesser overall economic importance than root knot and reniform nematodes. The sting nematode *Belonolaimus longicaudatus* is an aggressive pathogen of cotton in the sandy soils of south-eastern USA (Fig. 21.7). *B. longicaudatus* will not survive in soils with less than 85% sand content (Robbins and Barker, 1974). Feeding activities of this nematode cause much destruction of cortical tissues, resulting in severely stunted, necrotic root systems and similarly stunted shoot growth. Damage thresholds are low, 1–5 individuals/100 cm³ soil, and the severe damage to cotton by this nematode results in a carrying capacity of only 100 nematodes/100 g soil (Crow *et al.*, 2000). The sting nematode can be managed by rotation with tobacco (*Nicotiana tabacum*), *C. spectabilis* and *Tagetes minuta* (Good *et al.*, 1965). Tomerlin (1969) reported that various organic soil amendments suppressed *B. longicaudatus*, while a strain of the obligate endoparasitic bacterium *Pasteuria*, which is able to parasitize *B. longicaudatus*, has been reported (Giblin-Davis *et al.*, 2001).



Fig. 21.7. Severe root stunting of cotton due to *Belonolaimus longicaudatus*. (Photograph courtesy of W.T. Crow.)

Unidentified species of *Longidorus* and *Xiphinema* have been associated with cotton exhibiting poor growth and roots with symptoms of nematode damage in southern Africa (Bridge and Page, 1975).

Kenaf

Kenaf (*H. cannabinus*) is an important fibre crop in several countries with tropical or subtropical climates. *Meloidogyne arenaria*, *M. incognita* and *Meloidogyne javanica* are pathogens of kenaf in nearly all production regions, causing substantial galling of the roots. Kenaf is also a host for *H. columbus* (Koura *et al.*, 1987) and *H. magnistylus* (Lawrence and McLean, 1990), although yield losses due to these species have not been documented. Kenaf has been reported as a non-host for *R. reniformis* (Robinson *et al.*, 1998), a relatively poor host (Robinson and Cook, 2001) and a good host (Lawrence and McLean, 1992).

Several studies have examined the relationship between initial nematode population densities and yield of kenaf. McSorley and Parrado (1986) were able to relate root galling due to *M. incognita* with growth (height) using the

Seinhorst model, and observed a damage threshold of eight galls per root system. Di Vito *et al.* (1997) also related the growth of kenaf to *M. incognita* densities using the Seinhorst model, and found a damage threshold for shoot weight of 0.18 eggs and J2/cm³ soil with a relative minimum yield of 0.1. Zhang and Noe (1996) reported that the growth response of kenaf to infection by *M. arenaria* and *M. incognita* was similar.

Management with nematicides, crop rotation and the use of resistance/tolerance can reduce yield losses (Tahery *et al.*, 2011). Resistance to *M. incognita* and *M. javanica* in kenaf has been reported (Adegbite *et al.*, 2005, 2008). Tolerance also has been identified (Lawrence *et al.*, 1994; Cook and Scott, 1995). Effective rotation crops will vary depending on both the *Meloidogyne* species and race. Cotton, groundnut and soybean cultivars with high levels of resistance to various *Meloidogyne* spp. populations are potentially useful rotation crops.

Roselle

Roselle (*H. sabdariffa*) is frequently grown in tropical environments that are conducive to root knot and reniform nematodes. Roselle varies in

its susceptibility to *M. incognita*, *M. javanica* and *M. arenaria* (Minton *et al.*, 1970). Adeniji (1970) and Vawdrey and Stirling (1992) reported that several roselle accessions were resistant to *M. incognita* and *M. javanica*. In contrast, Heffes *et al.* (1991) reported that roselle (sorrel) cv. Red was severely galled and supported at least moderate levels of reproduction of *M. incognita*. Roselle yields were increased in fields infested with *M. arenaria* and *M. incognita* but not with *M. javanica*, when treated with nematicides (Adegbite *et al.*, 2008). The susceptibility of roselle to *R. reniformis* varies from low to moderate, depending on roselle genotype (Heffes *et al.*, 1990).

Jute

Two species of jute, *C. capsularis* and *C. olitorius*, are grown in several tropical regions as a fibre crop. Both jute species are hosts to *M. arenaria*, *M. incognita* and *M. javanica*, and can be severely galled by these nematodes. *Meloidogyne hapla* and *Meloidogyne thamesi* are reported parasitic on jute in China (Lin and Chen, 1992). Additionally, *H. indicus*, *Helicotylenchus* spp. and *R. reniformis* have been associated with crop damage in India (Mishra *et al.*, 1985). *H. indicus* and *Helicotylenchus* spp. feed endoparasitically in

the root cortex, resulting in necrosis and stunting of severely infected root systems.

Root knot nematodes are often involved with several soil-borne pathogens to cause disease complexes of jute. These pathogens include *Fusarium solani*, *Rhizoctonia bataticola*, *R. solani* and *Ralstonia solanacearum* (*Pseudomonas solanacearum*), causing a syndrome known as Hoogly wilt (Mandal and Mishra, 2001). Fibre yield loss due to combined infection of *M. incognita* and *R. solanacearum* was estimated to be as high as 35.6%, almost twice as much as with the nematode alone (Hazarika *et al.*, 2006).

Several approaches have been tested for the management of root knot nematodes on jute. Soil amendment with neem (*Azadirachta indica*) cakes at 0.5–1.0 kg/m² was effective against *Meloidogyne* spp. (Agbakli *et al.*, 1992; Chakraborti, 2001). A combination of crop stubble removal, rotation with rice (*Oryza sativa*) or wheat and amendment with poultry manure at 10 t/ha also improved jute yields in soils infested with *M. incognita* or *M. javanica* (Mishra *et al.*, 1987). Growing mustard in rotation with jute can suppress *M. incognita* soil densities (Khan and Banerjee, 2003). Whereas most cultivated jute cultivars appear to be susceptible to a range of *Meloidogyne* spp., some resistance (Laha *et al.*, 1995) or tolerance (Mishra and Chakraborti, 1987) has been described.

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22 Nematode Parasites of Spices and Medicinal Plants*

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Spices are strongly flavoured or aromatic substances of plant origin commonly used for seasoning and preserving foodstuffs. They consist of rhizomes, barks, leaves, fruit, seeds and other parts of plants. These plants belong to different families, genera and species. The bulk of the dry matter of their products consists of carbohydrates, volatile oils, fixed oils, proteins, tannins, resins, pigments and mineral elements. These constituents differ in their composition and content in different spices. Most of the spices are crops of the humid tropical regions. India is considered to be the home of spices from ancient times and produces a large proportion of all spices. There are innumerable biotic and abiotic problems on spice crops that affect production adversely, including plant parasitic nematodes, which can cause considerable damage to some of these crops. Nematode problems of chilli and garlic, which, depending on use, can be considered spices, are not included in this chapter as they are discussed under vegetables. Nematode problems of betel vine (*Piper betle*) and kava (*Piper methysticum*) are included in this chapter.

Traditional medicines derived from plant sources have gained credibility and have become an important aspect of herbal medicine systems for human health care. The herbal medicine system is widespread in China, India, Japan, Pakistan,

Sri Lanka and Thailand. Aroma compounds from botanical sources are used increasingly in the cosmeceutical, nutraceutical and processed food industries, due to growing public awareness of the risks involved in the use of synthetic additives. The plant retail for herbs and medicinal plants in the USA is estimated to have a turnover of approximately US\$1.6 billion annually. In Europe, about 400,000 t of medicinal plant material is imported from Asia and Africa yearly. The average market value of this plant material is estimated at US\$1 billion. Many of the raw materials used in the pharmaceutical industry come from medicinal plants produced on a global scale.

Black Pepper

Black pepper (*Piper nigrum*) is a branching and climbing perennial shrub belonging to the family Piperaceae and is cultivated in the hot and humid parts of the world. Vietnam, Indonesia, India and Brazil, contributing 31.9, 17.6, 16 and 10.2%, respectively, are the major pepper-producing countries in the world today. World production of pepper during 2015 was 407,158 t and covered an area of 471,300 ha (DASD, 2016). Its origin is considered to be in the hills of south-western India, where it is known as the

*A revision of the chapter by P.K. Koshy, S.J. Eapen and R. Pandey in the second edition.

'king of spices'. It is used in culinary seasonings, as a preservative for meat and other perishable foods, and in medicine. Piperine, the bite factor of pepper, is used to impart a pungent taste to brandy. Pepper oil is used in perfumery. The pepper vine can be propagated either vegetatively or by seed. Raising plants through cuttings is universally adopted. Two pepper vines entwined about a teak wood or concrete post, set in the field, is known as a 'pepper tree'. In India, live trees are used as supports (standards) for climbing pepper.

Nematodes on Black Pepper

Many plant parasitic nematodes have been reported on black pepper (Table 22.1), but the only two known to cause serious damage to the crop are *Radopholus similis* and *Meloidogyne* spp.

Radopholus similis

Association of the burrowing nematode *R. similis* with the yellows disease of pepper was first reported in 1936, and later by Van der Vecht (1950), who made extensive field studies and also demonstrated its pathogenicity under laboratory conditions. The nematode is notorious for being associated with the loss of 22 million pepper vines within 20 years in Bangka Island, Indonesia, due to 'yellows disease' (Christie, 1957, 1959). Subsequently, *R. similis* was reported from black pepper from India (D'Souza *et al.*, 1970; Kumar *et al.*, 1971; Venkitesan, 1972; Koshy *et al.*, 1978; Mohandas and Ramana, 1987c; Ramana *et al.*, 1987a; Ramana and Mohandas, 1989), Malaysia, Thailand (Sher *et al.*, 1969; Reddy, 1977) and Sri Lanka (Gnanapragasam *et al.*, 1985). The nematode is also involved in 'slow decline' or 'slow wilt' disease of black pepper in India, which is almost identical to pepper yellows in Indonesia (Van der Vecht, 1950; Mohandas and Ramana, 1987b); hence, they are dealt with together. Intensive surveys carried out on the role of plant parasitic nematodes in the slow decline disease complex of black pepper in India showed that high populations of *R. similis* occurred more frequently in slow decline disease-affected plants than in healthy plants. Discriminate analysis indicated the involvement of *R. similis* in the disease (Ramana *et al.*, 1987a).

Black pepper was introduced to Indonesia from Kerala, India (Nambiar, 1977), and it is quite likely that the burrowing nematode was also introduced along with the rooted cuttings of black pepper.

Symptoms of damage

The primary symptom of the slow decline disease is the appearance of pale yellow or whitish yellow drooping leaves on the vines. The number of such leaves increases gradually until large numbers of leaves, or even the entire foliage, becomes yellow. Yellowing is followed by shedding of leaves, cessation of growth and dieback symptoms (Fig. 22.1). The symptoms are very pronounced when soil moisture is depleted. In the very early stage of the disease in India, the symptoms may disappear with the onset of the south-west monsoon, resulting in an apparently healthy appearance of such plants in the following years because of new leaf growth and the shedding of yellowed leaves. This has often given a mistaken impression of the disease being caused by soil moisture stress rather than nematodes. However, within 3–5 years of initiation of yellowing, all the leaves are shed and death of the vine takes place, and hence the name 'slow decline' disease. In bearing vines, shedding of spikes (inflorescences) is a major symptom. Large numbers of shed spikes are seen at the base of affected vines. In large plantations, affected patches become conspicuous initially as yellowed plants, and later with large numbers of barren standards that have lost the vines, or standards supporting dead vines without any leaves. Young and old plants are affected and the replanted vines normally die within 2 years.

The tender thin, white feeding roots show typical orange- to purple-coloured lesions. Lesions are not seen clearly on older roots, being brown in colour. The root system exhibits extensive rotting, and the main roots are devoid of fine feeder roots that rot quickly. Extensive necrosis of larger lateral roots develops over time.

Biology and life cycle

The nematode penetrates roots within 24 h of inoculation, and the cells around the site of penetration become brown (Venkitesan and Setty, 1977). Nematodes do not enter the stelar portions of the root, but plugging of xylem vessels with a

Table 22.1. Plant parasitic nematodes associated with spice crops.

Nematode species	Black pepper	Cardamom	Ginger	Turmeric	Fennel	Fenugreek	Coriander	Cumin	Celery	Dill	Vanilla
<i>Criconea cardamomi</i>		•									
<i>Criconemella</i> spp.	•										
<i>Criconemoides</i> spp.	•		•	•	•	•	•		•		
<i>Discocriconemella limitanea</i>	•										
<i>Helicotylenchus</i> spp.	•	•	•	•		•	•				
<i>Hemicriconemoides</i> spp.	•	•	•	•		•					
<i>Hemicycliophora arenaria</i>						•					
<i>Hirschmanniella mucronata</i>							•				
<i>Hirschmanniella oryzae</i>							•				
<i>Hoplolaimus</i> spp.	•		•	•		•	•				
<i>Meloidogyne</i> sp.	•										
<i>Meloidogyne arenaria</i>		•	•		•	•					
<i>Meloidogyne hapla</i>			•		•		•		•	•	
<i>Meloidogyne incognita</i>	•	•	•	•	•	•	•	•	•		
<i>Meloidogyne javanica</i>	•	•	•	•	•	•	•	•	•	•	
<i>Meloidogyne piperi</i>	•										
<i>Pratylenchus</i> sp.	•										
<i>Pratylenchus brachyurus</i>	•		•								
<i>Pratylenchus coffeae</i>	•	•	•	•					•	•	
<i>Pratylenchus exilis</i>							•				
<i>Pratylenchus indicus</i>			•								
<i>Pratylenchus penetrans</i>					•				•		
<i>Pratylenchus pratensis</i>			•								
<i>Pratylenchus thornei</i>						•	•				
<i>Pratylenchus zeae</i>			•			•	•				
<i>Radopholus similis</i>	•	•	•	•		•	•				
<i>Rotylenchulus reniformis</i>	•	•	•	•		•	•	•			
<i>Rotylenchus</i> sp.	•	•		•							
<i>Scutellonema</i> sp.	•										
<i>Trophotylenchulus piperis</i>	•										
<i>Tylenchorhynchus</i> spp.	•				•	•					
<i>Tylenchulus semipenetrans</i>	•				•						
<i>Xiphinema</i> spp.	•		•	•			•			•	•



Fig. 22.1. A black pepper garden showing symptoms of slow decline disease. (Photograph courtesy of S.J. Eapen.)

gum-like substance has been reported (Freire and Bridge, 1985a). *R. similis* completes its life cycle within 25 days, in a temperature range of 25–28°C (Geetha, 1991). The black pepper isolate of the nematode is easily cultured on carrot discs at 25°C (Koshy, 1986b). The *R. similis* populations in Indonesia and Kerala (India) have a haploid number ($n = 4$) of four chromosomes and belong to the 'banana race' (Huettel *et al.*, 1984; Koshy, 1986b; Jasy, 1991; Ramana, 1992).

In India, the maximum nematode population in roots of pepper occurs between September and October and the minimum density between April and May (Ramana, 1986; Mohandas and Ramana, 1988). Low soil temperatures coupled with adequate soil moisture and availability of young tender roots help in the build-up of the population during September–October.

Other hosts

A large number of tree species such as coconut (*Cocos nucifera*), arecanut (*Areca catechu*), jack

fruit (*Artocarpus integrifolia*), mango (*Mangifera indica*), gliricidia (*Gliricidia maculata*), dadap (*Erythrina indica*), garuga (*Garuga pinnata*) and vatta (*Macaranga indica*) are used as live standards. Among these, coconut and arecanut are good hosts of *R. similis*. Crops such as banana, ginger and turmeric, which are susceptible to *R. similis*, are also intercropped with pepper.

Disease complexes

It has been speculated that yellows disease in Indonesia is caused by a nematode–fungus complex (Hubert, 1957; Bridge, 1978) involving *R. similis*, *Fusarium* spp. and possibly other fungi. There is little direct evidence to support the hypothesis. However, Freire (1982) showed that an Indonesian isolate of *R. similis* predisposed black pepper seedlings to attack by a weakly pathogenic isolate of *Fusarium solani*, causing severe root damage. In addition, Mustika (1992a,b) has clearly demonstrated that *R. similis* alone

caused growth reduction and yellow leaves with a stiff droop, but damage was more obvious when *R. similis* acted together with *F. solani*. Studies under simulated field conditions showed that *R. similis* and *Phytophthora capsici*, alone or in association, resulted in root rotting, leading to slow decline disease (Ramana *et al.*, 1992; Anandaraj *et al.*, 1996a,b).

Economic importance and population damage threshold levels

Slow decline was first reported from the Wynad area in Kerala as early as 1902, and Krishna Menon (1949) reported mortality of up to 10% of the vines due to the disease. Reduction in plant growth has been reported in sterile soil when 55-day-old rooted cuttings of black pepper in pots are inoculated with 2300 nematodes.

The onset of yellows disease in Sumatra, Indonesia, is correlated with *R. similis* populations of 2 nematodes/100 g of soil and 25 nematodes/10 g of roots, and *Meloidogyne* spp. populations of 47 nematodes/100 g of soil and 305 nematodes/10 g of roots (Mustika, 1978). Bridge (1978), however, stated that a low population of less than 310 nematodes/10 g of roots might not cause the disease alone. A population level of 250 nematodes/g of roots was constantly recorded with slow wilt-affected pepper vines in Kerala (Ramana, 1986). In pathogenicity tests, *R. similis* caused significant reduction in the growth and yield of black pepper (Mohan-das and Ramana, 1991). Black pepper vines of any age group are susceptible to this nematode (Ramana, 1992). Inoculation with *R. similis* alone reduced the growth rate of different cultivars of black pepper (Mustika, 1991).

Management measures

At present, there are no effective control measures for slow decline or pepper yellows. The price of black pepper is known to fluctuate greatly and, with a fall in prices, the farmer often loses interest in the crop and tends to neglect adoption of even standard agronomic practices. Control methods need to be adopted every year for black pepper, which is a perennial crop, especially under Indian conditions where live standards are used. The perennial multicropping systems involving coconut, arecanut, black pepper, betel

vine, banana, ginger, turmeric, etc., that have developed over many years on the west coast of south India are ideal situations where the burrowing nematode multiplies and causes extensive damage to all the susceptible crops. Black pepper, betel vine and banana are crops that succumb to nematode attack early. In later years, farmers abandon pepper cultivation in arecanut-based farming systems where arecanut is the live standard. Although the application of phorate at 3 g a.i./vine twice a year has been found to control *R. similis*, the high-density multispecies cropping pattern does not permit the use of nematicides, as most of the crops are export oriented and some products are consumed without any processing or cooking, such as banana, betel leaves, etc. This situation is complicated further because arecanut and coconut that are used as live standards are also very good hosts of *R. similis*, which warrants higher dosages and more frequent use of nematicides, especially under irrigated conditions.

CULTURAL: symptoms of slow wilt and pepper yellows are known to be ameliorated with mulching. Pasril (1976) has recorded an 18% reduction in disease incidence on Bangka Island, Indonesia, after mulching. He also observed a reduction in disease symptoms after the application of nematicide, with a corresponding increase of yield in the first year of treatment. The addition of chopped leaves of *G. maculata* (10 g/kg of soil) as green manure reduced populations of *R. similis* and increased plant growth (Jasy and Koshy, 1992).

De Waard (1979) suggested the application of fertilizers at a per hectare dose of 400 kg N, 180 kg P, 480 kg K, 425 kg Ca and 112 kg Mg in combination with a mulch for effective control of yellows disease in Bangka, Indonesia. Mustika *et al.* (1984) also reported remission of disease severity when fertilizers were applied to infected vines. Furthermore, foliar yellowing and necrosis of distal ends of laminae of slow wilt-affected vines in Kerala, India, were attributed to N and K deficiencies (Wahid *et al.*, 1982).

RESISTANCE AND TOLERANCE: a number of black pepper germplasm accessions, including wild types, have been screened against *R. similis* by several workers (Venkitesan and Setty, 1978; Jacob and Kuriyan, 1979a; Koshy and Sundararaju, 1979; Leong, 1986; Paulus *et al.*, 1993; Eapen

et al., 2011). The wild collection Vittal No 430, *Piper hymenophyllum* and *Piper attenuatum*, recorded less than 30% root reduction and a 1.5-fold nematode population increase. The hybrid pepper cultivar Panniyur-I recorded 91.4% root reduction and a 7.6-fold nematode increase (Venkitesan and Setty, 1978). However, a local cultivar at Peringamala in Kerala was not invaded by *R. similis* (Jacob and Kuriyan, 1979b). In Sri Lanka, a black pepper cultivar, PW 14, was immune to *R. similis* (Gnanapragasam, 1989). No resistance or tolerance has been found on screening cultivated and wild germplasm, intercultural hybrids or open pollinated seedlings, except for *Piper colubrinum*, which is now used widely as a rootstock to graft cultivated pepper plants (Ramana *et al.*, 1987b; Ramana, 1992). However, Eapen *et al.* (2011) could identify a few cultivated germplasm accessions and hybrids resistant to *R. similis* that succumbed to nematode attack on prolonged field testing.

CHEMICAL: a number of non-fumigant nematicides have been found effective in reducing *R. similis* populations on black pepper under greenhouse and field conditions (Venkitesan, 1976; Mustika and Zainuddin, 1978; Venkitesan and Setty, 1979; Ramana, 1986; Mohandas and Ramana, 1987a; Lokesh and Gangadharappa, 1995; Sundararaju and Sudha, 1998). Many of them are either not available in the market or lack label claim. The chances of rehabilitating severely affected vines by the application of nematicides are low, because of the heavy damage already caused to the root system and the inability of such plants to put out new roots for quick rejuvenation. A list of nematicides that might be useful in black pepper is listed in Chapter 23, this volume. Nematicides are highly toxic to humans and the environment; therefore, caution needs to be taken in using these products in the field. There are also differences in registration for the use of these products on a per country basis, and this has to be determined before use in the field.

BIOLOGICAL: there have been very few successful attempts to control *R. similis* by using any of the fungal biological control agents, probably due to the migratory endoparasitic nature of this nematode (Geetha, 1991; Ramana, 1994). The mycorrhizal fungus *Glomus fasciculatum*

suppressed burrowing nematode infestation (Anandaraj *et al.*, 1996c). In a field study, Koshy *et al.* (2003) proved the efficacy of arbuscular mycorrhizal fungi *Purpureocillium lilacinum* (syn. *Paecilomyces lilacinus*) and *Pasteuria penetrans* individually or in combinations in enhancing plant growth and suppressing nematode multiplication. Recently, rhizobacteria and endophytic bacteria that suppressed *R. similis* infesting black pepper have been identified in laboratory and greenhouse studies (Beena *et al.*, 2003; Aravind *et al.*, 2009, 2010). Some of the promising bacteria that suppress *R. similis* are *Pseudomonas putida* (Aravind *et al.*, 2010; Sheoran *et al.*, 2015), *Bacillus megaterium* (Aravind *et al.*, 2010) and *Curtobacterium luteum* (Aravind *et al.*, 2010). Pre-plant bacterization with such promising bacteria could produce nematode-free plantlets in black pepper nurseries (Aravind *et al.*, 2012). Soil application of consortia formulation of *Pseudomonas fluorescens* and *Bacillus subtilis* significantly reduced nematodes and enhanced the growth and yield of black pepper in field trials (Jonathan *et al.*, 2012). The deployment of such efficient bioagents is acceptable in organic agriculture provided the microorganisms are registered for commercial use in the country concerned.

Summary of management measures

Integrated methods of nematode management that can be suggested are:

- Planting of nematode-free rooted cuttings raised in nursery mixture sterilized with steam, solar heat or fumigants.
- Uprooting of affected vines and replanting after a period of 9–12 months.
- Use of non-living supports or standards.
- Exclusion of *R. similis*-susceptible trees as standards for trailing black pepper vines, and exclusion of susceptible intercrops such as banana, ginger and turmeric.
- Application of a tested nematicide to the vine with the onset of the monsoon and again after 3 months. The nematicide may be applied after removing the topsoil without causing damage to the roots, followed by replacement of the soil. The susceptible intercrops, e.g. banana, may also be treated with nematicides.

- Application of organic amendments, such as 200 g of neem oil cake (*Azadirachta indica*), green foliage (3–5 kg) or farmyard manure (1 kg) per vine.
- Earthing-up after the application of nematocides, NPK fertilizers and organic amendments in September/October.

Methods of diagnosis

The presence of nematodes and their association with disease can be diagnosed by soil sampling at a distance of 25–50 cm from the base of the vine at a depth of 20–30 cm. A soil sample of 200 cm³ and root sample of 0.5–1.0 g of thin, tender feeder roots should be taken to obtain maximum nematode population estimates (Koshy, 1986b, 1987a, 1988). Infested roots showing lesions and rotting may be split longitudinally and cut to a length of 1–2 cm. When such roots are submerged in water contained in Petri dishes or shallow pans and incubated at 20–25°C, 50% of nematodes are released in 72 h. A rapid and reliable single-tube duplex PCR-based method to detect *P. capsici* and *R. similis* in infected roots of black pepper has been reported (Aravind *et al.*, 2011).

Meloidogyne

The root knot nematode *Meloidogyne* sp. was the first nematode to be recorded on black pepper (Delacroix, 1902) in Cochin, China. In 1906, Butler reported root knot nematodes from black pepper in Wynad, Kerala (India). *Meloidogyne javanica* and *Meloidogyne incognita* have been reported from India, Brazil, Sarawak, Borneo, Cochin-China (Vietnam), Malaysia, Brunei, Cambodia, Indonesia, the Philippines, Thailand and Vietnam (Winoto, 1972; Castillo, 1974; Lordello and Silva, 1974; Ichinohe, 1975; Reddy, 1977; Freire and Monteiro, 1978; Kueh and Teo, 1978; Sundararaju *et al.*, 1979a; Ramana and Mohandas, 1983) and *Meloidogyne arenaria* from Sri Lanka (Lamberti *et al.*, 1983). A further species, *Meloidogyne piperi*, has been described from Kerala, India (Sahoo *et al.*, 2000).

Symptoms of damage

A gradual decline, characterized by unthrifty growth and yellowing of leaves, is the prominent

symptom. The leaves of vines infested with *Meloidogyne* spp. exhibit dense yellowish discoloration of the interveinal areas, making the leaf veins quite prominent with a deep green colour, whereas the leaves of vines infested with *R. similis* show uniform pale yellow or whitish discoloration and typical drooping (Ramana *et al.*, 1994). Kueh (1990) observed that the leaves of root knot nematode-infested vines were held inward and upward, and would then drop. *M. incognita* infestation reduced the uptake of nutrients such as P, K, Zn, Mn and Cu (Ferraz *et al.*, 1988). The total chlorophyll content of the leaves was reduced significantly by root knot nematodes, leading to senescence of the leaves (Ferraz and Lordello, 1989). Root systems become heavily galled and the adult females with egg masses are generally enclosed deep within the root tissue (Fig. 22.2) (Ramana, 1992; Ramana *et al.*, 1994). In the cv. Panniyur I, the galls are smooth and larger in size compared with the small galls with exposed egg masses, giving a pitted, rough appearance to the roots of cv. Karimunda.



Fig. 22.2. Black pepper root system showing galling due to root knot nematode infestation. (Photograph courtesy of S.J. Eapen.)

Other hosts

Among the commercially used standards, *Oroxylum indicum*, *Erythrina lithosperma*, *Ceiba pentandra* and *Bombax malabaricum*, are highly susceptible to root knot nematodes, whereas *G. pinnata* and *M. indica* are not susceptible. *E. indica* and *Gliricidia sepium* are less susceptible (Koshy *et al.*, 1977). A large number of weeds often found in pepper gardens have also been recorded as hosts of the root knot nematode (Ramana, 1986).

Disease complexes

Meloidogyne spp. does not significantly enhance the susceptibility of pepper vines to foot rot (Holliday and Mowat, 1963). *M. incognita* and *F. solani* were found to be associated with black pepper vines in Paraiba State, Brazil. Infested plants showed wilting, yellowing of leaves, rotting of stems and roots and cracking of stems; cracked stems 5–10 cm above the soil surface were heavily infected. Joint attack by *R. similis*, *M. incognita* and *Fusarium* sp. caused severe necrosis in the stelar part, and resulted in the formation of tyloses that blocked the xylem (Mustika, 1984). Both organisms together were also found to do more harm than either of them alone in other countries (Lopes and Lordello, 1979; Sheela and Venkitesan, 1990; Mustika, 1991, 1992a,b; Zhou and Chi, 1993). *F. solani* and *M. incognita* were of common occurrence in black pepper nurseries in Quang Tri province in Vietnam (Thuy *et al.*, 2013). Winoto (1972) reported increased susceptibility of *M. incognita* and *M. javanica* infested pepper cv. Kuching to *Phytophthora* infection in Malaysia. In India, black pepper plants also showed wilting symptoms quicker when root knot and burrowing nematodes were inoculated first, followed by *P. capsici* (Ramana *et al.*, 1992; Anandaraj *et al.*, 1996a,b). *Rotylenchulus reniformis* was found to inhibit the multiplication of *M. incognita* and the resultant damage on black pepper in autoclaved soil in pots under greenhouse conditions in Brazil (Ferraz and Sharma, 1979). The root gall development and population build-up of *M. incognita* were suppressed in black pepper on inoculation with *R. similis* in succession in sterile soil under pot conditions (Sheela and Venkitesan, 1981).

Economic importance and population damage threshold levels

As much as 91% root knot nematode infestation was reported from Para, Brazil (Ichinohe, 1975), and Kerala, India (Ramana and Mohandas, 1987b; Ramana *et al.*, 1987a). An initial population of ten juveniles per rooted cutting reduces growth by 16%, while a maximum of 50% reduction is observed at an inoculum level of 100,000 over a period of 1 year in sterile soil under potted conditions (Koshy *et al.*, 1979b). *M. incognita* was found highly pathogenic at 100–10,000 juveniles/seedling (Freire and Bridge, 1985c; Mohandas and Ramana, 1991). In Indonesia, yellow symptoms appeared on plants with *Meloidogyne* spp. at population levels of 47 nematodes/100 g of soil and 305 nematodes/10 g of roots (Mustika, 1978).

Management measures

Root knot infestation in black pepper nurseries has been a serious problem in several government nurseries in Kerala, India. Fumigation of nursery potting mixture with methyl bromide is effective in checking the infestation (Koshy, 1974, 1986a; Mohandas and Ramana, 1987a).

CULTURAL: growing of the non-host cover plant siratro (*Macroptilium atropurpureus*) in the interspace and mulching with Guatemala grass (*Imperata cylindrica*) are recommended to reduce populations of *M. incognita* on black pepper in the Amazonian region (Ichinohe, 1980, 1984). Mulching the basins with *Gliricidia* leaves reduced root knot nematodes in Sri Lanka (Ratnasoma *et al.*, 1991). The application of botanicals such as neem oil cake can also reduce root knot nematodes (Ramana *et al.*, 1992). In Vietnam, it is suggested that the management of root knot nematodes be initiated during the first half of the dry season (Thuy *et al.*, 2012b).

RESISTANCE AND TOLERANCE: among the seven popular cultivars screened, the hybrid cultivar Panniyur-I was the most susceptible, and the cv. Valiakaniakadan was the least susceptible (Koshy and Sundararaju, 1979). The intensity of *M. incognita* damage was less in cultivar Karimunda compared with that of Panniyur-I

(Mohandas and Ramana, 1983). Of eight cultivars screened against *M. incognita*, Kalluvalli, Balancotta, Karimunda, Narayakodi and Pada-pan had fewer galls than Panniyur-I, Cheriya-kanikadan and Kottanadan (Jacob and Kuriyan, 1979a). A total of 101 cultivars, 74 accessions of wild *Piper* sp. and 140 intercultural hybrids were screened against *M. incognita*, of which one cultivar, CLT-P-812, was found resistant (Ramana and Mohandas, 1986, 1987b; Koshy, 1987b). This cultivar was released as 'Pournami' for cultivation in root knot-infested areas (Ravindran *et al.*, 1992). Some of the wild related species of *Piper* were resistant to root knot nematodes (Ramana, 1992; Paulus *et al.*, 1993). Screening of 431 black pepper germplasm accessions yielded several resistant genotypes, out of which Acc. 1047 (IC No 316602) and Acc. 1090 (IC No 316635) were free of root knot nematode infestation under field conditions (Eapen *et al.*, 2011). In addition, an open progeny of a black pepper hybrid, 04-HP 1533 (2), showed resistance towards *M. incognita* (Bhai *et al.*, 2010).

Infection by nematodes is known to cause biochemical changes in plants (Eapen *et al.*, 1999a). The cv. Cingapura recorded high concentrations of total phenols on inoculation with 6000 *M. incognita* juveniles/pot 95 days after planting, although no resistance was shown (Ferraz *et al.*, 1984). Changes in levels of amino acids, organic acids and sugars in *M. incognita*-inoculated plants compared with uninfected plants were reported by Freire and Bridge (1985b).

CHEMICAL: several nematicides have been found to be effective in reducing root knot nematode populations on black pepper (Kueh and Teo, 1978; Ichinohe, 1980, 1984; Venkitesan and Jacob, 1985; Leong, 1986; Ratnasoma *et al.*, 1991), but information on their practical use is limited. Many non-fumigant and fumigant nematicides are either no longer registered or have restrictions for use on certain food crops. New nematocidal compounds with different modes of action have also been developed (see Chapter 23, this volume).

BIOLOGICAL: nematode-free cuttings could be raised by incorporating a biological control agent in the potting mixture. A number of organisms have been tested and found to be effective in reducing root knot nematodes. Promising

among these are *P. lilicinium* (syn. *P. lilacinus*) (Freire and Bridge, 1985d; Ramana, 1994; Sosamma and Koshy, 1997; ChenGuan *et al.*, 2012), *Pochonia chlamydosporia* (syn. *Verticillium chlamydosporium*) (Freire and Bridge, 1985d; Sreeja *et al.*, 1996), *P. penetrans* (Ratnasoma *et al.*, 1991; Sosamma and Koshy, 1997; XiaoXiao *et al.*, 2015), *Bacillus* spp. (Sheela *et al.*, 1993; Devapriyanga *et al.*, 2012) and *Pseudomonas* spp. (Eapen *et al.*, 1997; Devapriyanga *et al.*, 2012). In the field, *P. chlamydosporia* and *P. penetrans* significantly suppressed root knot nematodes and increased the yield of black pepper (Eapen *et al.*, 2009). A number of rhizosphere and endophytic bacteria that are antagonistic to root knot nematodes have been isolated recently (Beena *et al.*, 2001; Aravind *et al.*, 2009). Black pepper plants pre-inoculated with arbuscular mycorrhizal fungi such as *G. fasciculatum*, *Glomus etunicatum*, *Glomus mosseae* and *Gigaspora margarita* recorded a significant increase in growth even in the presence of root knot nematodes (Sivaprasad *et al.*, 1990, 1992; Anandaraj *et al.*, 1991). The combined application of *P. fluorescens* and *B. subtilis* has enhanced the activities of plant defence enzymes, reduced root knot nematodes significantly and increased the growth and yield of black pepper under field conditions (Devapriyanga *et al.*, 2011; Jonathan *et al.*, 2012).

Other nematodes of black pepper

The other nematodes that have been found to be associated with black pepper (see Table 22.1) in various countries are considered to be of minor economic importance (Timm, 1965; Sher *et al.*, 1969; Castillo, 1974; Sharma and Loof, 1974; Ichinohe, 1975; Reddy, 1977; Bridge, 1978; Sundararaju *et al.*, 1979b; Dasgupta and Rama, 1987; Rama, 1987; Ramana and Mohandas, 1987a; Thuy *et al.*, 2012a; Duong *et al.*, 2014; Pervez and Eapen, 2015). *Trophotylenchulus piperis* has been reported as a widespread parasite of black pepper roots in South India (Mohandas and Ramana, 1982; Mohandas *et al.*, 1985; Ramana and Mohandas, 1987a, 1989; Sundararaju *et al.*, 1997). *T. piperis* completed its life cycle on black pepper roots within 55 days at a temperature of 24–32°C (Sundararaju *et al.*, 1995). Feeding of this nematode on black pepper roots caused drying and shrinkage of cells in the vicinity of infection (Ramana and Eapen, 1997).

Future prospects

The incorporation of crop rotation systems designed to reduce root knot densities in soil, avoiding susceptible live supports or standards and using resistant cultivars where present, in an integrated nematode management system with minimum or no nematicide application, should be the main thrust of research to increase black pepper yield in areas infested with damaging nematodes. The deployment of promising biocontrol agents like *P. chlamydosporia*, *P. penetrans* and a number of rhizosphere and endophytic bacteria may be a possible means of managing nematodes in black pepper gardens in the future, should they become economically acceptable products.

Cardamom

Cardamom is a fruit (capsule) of the plant *Elettaria cardamomum*, belonging to the family Zingiberaceae. It is a perennial plant having an underground stem (rhizome) with aerial shoots. A mature cardamom plant may measure about 2–4 m in height. Flowers are borne on panicles that emerge directly from the swollen base of the aerial shoot. The fruits are small, trilocular capsules containing 15–20 seeds. Cardamom, known as the ‘queen of spices’, has its origin in the evergreen rainforests of south India. Cardamom is basically a shade-loving plant. India and Guatemala are the main producers and exporters of cardamom. Tanzania, Sri Lanka, El Salvador, Vietnam, Laos, Kampuchea and Papua New Guinea are also cardamom growers. The area under cardamom cultivation in India during 2014/15 was 69,970 ha, and the total production was 18,000 t (DASD, 2016). Cardamom is used for flavouring various food preparations, confectionery, beverages, liquors and medicines. Cardamom can be propagated through seedlings, as well as suckers. Suckers are better suited for gap filling and multiplication of selected high-yielding types.

Nematodes of Cardamom

Nematological investigations on this crop have been undertaken in India, where a number of

plant parasitic nematodes have been found (see Table 22.1). The most important nematode problem is caused by the root knot nematodes, *Meloidogyne* spp., although the lesion nematode *Pratylenchus coffeae* and the burrowing nematode *R. similis* are also known to cause root rotting (D'Souza *et al.*, 1970; Kumar *et al.*, 1971; Khan and Nanjappa, 1972; Viswanathan *et al.*, 1974; Sundararaju *et al.*, 1979b). The reniform nematode, *R. reniformis*, has also been recorded on cardamom (Eapen, 1995a).

Meloidogyne

Widespread occurrence of root knot nematodes *M. incognita* and *M. javanica* has been reported in cardamom nurseries and plantations in India (Kumar *et al.*, 1971; Koshy *et al.*, 1976; Ali and Koshy, 1982a; Ali, 1985, 1986a; Raut and Pande, 1986).

Symptoms of damage

Heavy root knot nematode infestation in mature plants in a plantation causes stunting, reduced tillering, yellowing, premature drying of leaf tips and margins, narrowing of leaf blades, delay in flowering, immature fruit drop and reduction in yield. Unlike several other plant species, galling of roots is not a conspicuous symptom on mature plants. The infested roots, however, exhibit a ‘witch’s broom’ type of excessive branching (Fig. 22.3).



Fig. 22.3. Galling and abnormal branching in cardamom roots infested with *Meloidogyne* sp. (Photograph courtesy of S.J. Eapen.)

In the primary nurseries, more than 50% of the germinating seeds do not emerge as a consequence of infection of the radicle and plumule by the second-stage juveniles of the root knot nematode. The infested seedlings at the two-leaf stage show marginal yellowing and drying of leaves and severe galling of roots. On transplantation to a secondary nursery, they exhibit curling of the unopened leaves. These leaves mostly emerge after the breaking open of the pseudostem. Up to 40% of such seedlings do not establish in the secondary nursery. In secondary nurseries, the infested plants are stunted and yellowed, with poor tillering, drying of leaf tips and margins, and heavy galling of roots (Ali and Koshy, 1982a; Eapen, 1995b). Young seedlings are more susceptible to root knot nematode attack than mature plants, and galling is more prominent in seedlings (Eapen, 1992). Patches of stunted and weak plants with narrow leaves are a common symptom of nematode infestation in cardamom plantations (Eapen, 1994, 1995b).

Survival and means of dissemination

The heavily shaded, hot, humid atmosphere and continuous availability of soil moisture prevalent in cardamom plantations are congenial conditions for the multiplication of root knot nematodes. Root knot nematode population dynamics in cardamom plantations are influenced by rainfall, soil moisture, soil temperature and crop phenology. As a result, the root knot nematode population is generally high during the post-monsoon period between November and January (Eapen, 1993). The nematodes are disseminated through infested seedlings and rhizomes used for propagation. Most plantations have their own permanent nursery sites situated in areas having easy access to water sources such as forest streams.

Other hosts

A large number of annual weeds present in cardamom plantations and the common shade trees, *E. indica* and *E. lithosperma*, are hosts of root knot and help in the build-up of nematode populations (Muniappan, 1993).

Disease complexes

The incidence of rhizome rot and damping-off diseases caused by the fungus *Rhizoctonia solani* increases in the presence of *M. incognita* in

nurseries (Ali, 1986b; Eapen, 1987; Ali and Venugopal, 1992, 1993). The root knot nematode population was found to be 5–10 times higher in virus disease-affected cardamom plants than in healthy plants (Ali, 1989).

Economic importance

A yield loss of 32–47% due to root knot has been reported from the results of a nematicide experiment (Ali, 1985, 1986b). Microplot studies under simulated field conditions showed 46.6% yield loss at an initial inoculum level of 4 nematodes/100 ml of soil (Eapen 1987, 1994).

Management measures

Nematological investigations have helped in creating a general awareness among planters, as well as administrators, that the root knot nematode is a major limiting factor. However, planters have not yet adopted the recommended control measures. No resistance to root knot nematodes has been found, and the popular cardamom cvs. Malabar, Mysore and Vazhuka are all susceptible (Hegde *et al.*, 1993; Eapen, 1995b).

It is advisable to change nursery sites every year, but this is not always practicable in view of the difficulties involved in obtaining suitable sites having facilities for irrigation. Hence, disinfection of the nursery beds needs to be carried out every year. Disinfection of nursery beds with fumigant nematicides was found effective in controlling root knot infestation in both primary and secondary nurseries (Ali and Koshy, 1982b). Similarly, the efficacy of several granular nematicides has been proved by several worker's nurseries (Koshy *et al.*, 1979a; Jacob and Chandrasekharan, 1984; Ali, 1984, 1986b, 1986c, 1987; Eapen, 1995b).

As stated above, many of the formerly recommended nematicides are no longer considered safe for use, due to toxicity and environmental risks. Newer compounds that have been registered for use on spices crops need to be tested for efficacy and used in the future.

Cardamom nurseries are ideal for practising biological control. There are reports that *G. margarita* and *G. fasciculatum* reduced *M. incognita* infestation and enhanced the growth and vigour of seedlings (Thomas *et al.*, 1989). *P. lilacinum* reduced root knot nematodes by 48.5–57% in pot culture studies and by 19.7%

in field studies (Eapen, 1995b; Eapen and Venugopal, 1995). Some native isolates of *Trichoderma harzianum* and other *Trichoderma* spp. are potential antagonists of root knot nematodes. The reduction of root knot nematode infection by this fungus has been clearly shown in the laboratory and greenhouse, and also in cardamom nurseries (Eapen *et al.*, 2000a,b).

Ginger

Ginger is the rhizome or underground stem of *Zingiber officinale*, a herbaceous perennial belonging to the family Zingiberaceae. Although the country of origin is not known with certainty, it is presumed to be either India or China. It is grown in many countries of the tropics and subtropics, and is used widely in food, beverages, confectionery and medicines. India is the largest producer of dry ginger in the world, contributing about 31.9% of the world's production. In India, the total area under cultivation during 2014/15 was 141,650 ha and the total production was 760,310 t (DASD, 2016). The other ginger-producing countries are Nepal, Nigeria, China, Indonesia, Thailand, Bangladesh, Japan, Cameroon, the Philippines, Sri Lanka and Australia.

Ginger is propagated by seed rhizomes or setts. Seed rhizomes are cut into small pieces of 2.5–5 cm length, weighing 20–25 g each, having one or two good buds. It is grown either as a monocrop or as an intercrop in many farming systems. In India, the mulching of ginger beds with green leaves is a traditional practice to enhance the germination of seed rhizomes and the conservation of soil moisture. The first mulching is done at the time of planting itself, with green leaves at 10–12 t/ha, and repeated with 5 t/ha, 40–90 days after planting, immediately after weeding and the application of fertilizers.

Nematodes of Ginger

Plant parasitic nematodes belonging to 17 genera have been reported on ginger (Colbran, 1958; Reddy, 1977; Sundararaju *et al.*, 1979b; Rama and Dasgupta, 1985; Kaur, 1987; Ramana and Eapen, 1998); the most important parasites are *Meloidogyne* spp., *R. similis* and *P. coffeae*. In

Kerala, *M. incognita* and *R. similis* were the major nematode species found in the rhizosphere of ginger (Mammen, 1973; Charles, 1978; Sheela *et al.*, 1995). *R. reniformis* and *M. incognita* were the dominant plant parasitic nematodes associated with ginger in Orissa (Routaray *et al.*, 1987b). The most prominent nematode pests of ginger in Sikkim (Srivastava *et al.*, 1998) and Himachal Pradesh (Kaur *et al.*, 1989; Khan and Makhnotra, 1998) were *M. incognita* and *P. coffeae*, while in Madhya Pradesh *M. incognita* was the predominant nematode species (Vadhera *et al.*, 1998a). *M. arenaria* was also reported from Himachal Pradesh (Kaur and Sharma, 1988). In West Bengal, *R. reniformis*, *Hoplolaimus indicus* and *P. coffeae* recorded the highest relative density in ginger rhizosphere (Rama and Dasgupta, 1998, 2000).

Meloidogyne

Nagakura (1930) in Japan was the first to report *Meloidogyne* sp. on ginger, and subsequently the species *M. arenaria*, *Meloidogyne hapla*, *M. incognita* and *M. javanica* have been reported as parasites of ginger in various countries. A novel root knot nematode species parasitizing ginger, *Meloidogyne thailandica*, has been reported from Thailand (Handoo *et al.*, 2005).

Symptoms of damage

The root knot nematodes cause galling and rotting of roots and underground rhizomes. The second-stage juveniles of *M. incognita* invade the rhizome through the axils of leaf sheaths in the shoot apex. In fibrous roots, penetration occurs in the area of differentiation, and in fleshy roots, the entire length of the root is invaded. In both fleshy and fibrous roots, the nematode develops to maturity in 21 days, but in rhizomes it requires 40 days at 30°C (Cheng and Tu, 1979). Galls are formed on the fibrous roots. Abnormal xylem and hyperplastic parenchyma are observed in all infested tissue except rhizome meristems. Extensive internal lesions are formed in the fleshy roots and rhizomes. Wound cork around the lesions is suberized only in old rhizomes after harvest (Huang, 1966; Shah and Raju, 1977). Root knot nematode infection reduced the concentration of microelements like Fe and Zn, and increased B, Mn and Cu in leaves,

resulting in yellow leaves and prematurely senile and thin rhizomes. Plant hormones such as gibberellic acid (GA3), indole acetic acid (IAA) and zeatin riboside (ZR) were decreased significantly in infected leaves, due to nematode infection (YanYin *et al.*, 2004a,b). Infested rhizomes have brown, water-soaked areas in the outer tissues, particularly in the angles between shoots. Nematodes continue to develop after the crop has matured and been harvested, and induce breakdown of the seed rhizomes. Heavily infested plants are stunted, poorly tillered and have chlorotic leaves with marginal necrosis. The affected ginger plants mature, dry faster and die sooner than the healthy plants, leaving a poor crop stand at harvest. Infested rhizomes serve as a source of infection and means of dissemination.

Disease complexes

The incidence of rhizome rot of ginger caused by *Pythium aphanidermatum* is reported to be severe when rhizomes are infested with nematodes such as *M. incognita* and *P. coffeae* (Dohroo *et al.*, 1987). However, Doshi and Mathur (1987) could not observe any interaction with these two organisms. Similarly, there was also no interaction between *M. incognita* and *Pythium myriotylum* (Lanjewar and Shukla, 1985). Recent studies have shown that ginger plants inoculated with root knot nematodes developed disease symptoms earlier when inoculated with *P. aphanidermatum* (Ramana *et al.*, 1998). Bacterial wilt of ginger caused by *Ralstonia solanacearum* was also shown to be influenced by *M. incognita* (Samuel and Mathew, 1983; Das and Swain, 2014); however, there are contradictory reports on the subject (Ramana *et al.*, 1998).

Other hosts

Most of the weeds that are present in ginger-growing areas are known hosts of root knot nematodes.

Economic importance and population damage threshold levels

In Queensland, Australia, severe infestation of rhizomes reduces yields by 57%, as determined by fumigation (Pegg *et al.*, 1974). Treatment of infested soil with a mixture of 1,2-dichloropropane and 1,3-dichloropropene (D-D) before planting

nematode-free seed rhizomes has increased yields by 80%. *M. incognita* is distributed widely in ginger fields in India, and causes a loss of 46.4% (Charles, 1978). A reduction of 74% in rhizome weight has been recorded with an initial inoculum level of 10,000 nematodes/plant over a period of 6 months under potted conditions (Sudha and Sundararaju, 1986).

Both *M. incognita* and *M. hapla* cause significant reduction in shoot length and shoot and root weight following inoculation with root knot nematodes. The economic threshold level of this nematode varied from 2 nematodes/g of soil to 50 juveniles/100 ml of soil (Parihar and Yadav, 1986; Sudha and Sundararaju, 1986; Kaur, 1987; Routaray *et al.*, 1987a). At higher initial inoculum levels, *M. incognita* and *M. hapla* cause partial or complete withering of aerial shoots. Typical symptoms of drying and twisting of leaves were observed with *M. arenaria* (Kaur, 1987).

Significant damage is noticeable at 0.5 and 1.25 nematodes/g of soil and above in sterilized soil under potted conditions. The fibrous roots are very much reduced at 2 nematodes/g of soil (Parihar, 1985; Routaray *et al.*, 1987a). Ginger treated with carbofuran at 1 kg a.i./ha showed an increase of 20% in yield (Makhnotra and Khan, 1997). In another study, an avoidable yield loss of 43% was observed at an initial population level of 166 *M. incognita* juveniles/250 g of soil (Sheela *et al.*, 1995).

Management measures

Being an export-oriented crop, the nematodes of ginger have to be managed in an eco-friendly manner. Besides, as ginger is consumed raw, nematicides should be used with extreme care. A careful blend of the following measures may provide adequate management of the nematode problems in this crop.

PRODUCTION OF NEMATODE-FREE PLANTING MATERIAL: since the seed rhizome generally harbours nematodes, the selection of seed rhizomes is very critical for the management of nematodes. Nematode-free planting material should be selected from fields of known history. The control schedule for *M. javanica* involving the use of clean seed and a ginger-taro-fallow rotation has been recommended in Fiji (Haynes *et al.*, 1973).

In vitro ginger plantlets are used to solve root knot nematode problems in South Africa. Hot water treatment of seed rhizomes at 50–55°C, preferably 51°C, for 10 min was found to reduce the nematode incidence in ginger (Colbran and Davis, 1969; Anon., 1971; Bandyopadhyay and Bhattacharya, 2012). Disinfestation of rhizomes was also achieved by hot water treatment at 45°C for 3 h (Vadhera *et al.*, 1998a,b; 2002).

ORGANIC AMENDMENTS: mulching or applying well decomposed cattle or poultry manure, compost or neem oil cake reduced nematode build-up (Colbran, 1974; Kaur, 1987; Stirling, 1989; Dohroo *et al.*, 1994; Mohanty *et al.*, 1995; Vadhera *et al.*, 1998b). Growing under sawdust mulch reduced root knot nematode infestation in Australia (Pegg *et al.*, 1974). Pre-plant application of neem cake at 1 t/ha reduced *M. incognita* and increased yield (Mohanty *et al.*, 1995). Ginger plots mulched with mahaneem leaves at 2.5 kg/m² reduced root knot (Das, 1999). Studies in Australia have suggested that root knot on ginger can be controlled by alternating ginger with a green manure crop and applying at least 150 m³/ha/year of poultry manure (Stirling, 1989; Stirling and Nikulin, 1998). Intercropping bell pepper with ginger significantly reduced both *P. penetrans* and *M. incognita* and improved the yield of ginger (Sharma and Bajaj, 1998). The incorporation of organic materials fortified with biocontrol agents such as *Trichoderma* spp., *P. lilacinum*, *P. chlamydosporia*, etc., is another option to prevent nematode build-up (Eapen and Ramana, 1996).

HOST RESISTANCE: there are very few reports on resistance in ginger to root knot. In a preliminary evaluation, a few lines of ginger (Accession Nos 36, 59 and 221) were found to be resistant to *M. incognita* (Eapen *et al.*, 1999b). One of these has been recommended for release as IISR Mahima (Sasikumar *et al.*, 2003).

CHEMICAL CONTROL: soil fumigation or the application of non-fumigant nematicides to the soil or as dip treatment have been recommended in the past for the control of nematodes of ginger (Colbran, 1972; Willers, 1985, 1991; Kaur, 1987; Mohanty *et al.*, 1995; Kang *et al.*, 2012).

BIOLOGICAL CONTROL: a large number of bacterial and fungal isolates of biocontrol agents were isolated from ginger fields through random surveys (Ramana *et al.*, 2002). Many of the fungal isolates parasitized root knot nematode egg masses and suppressed their egg hatching. Toxic metabolites of some of them caused mortality of second-stage juveniles, in addition to direct parasitism. These studies indicated that five biocontrol agents, namely *P. chlamydosporia*, *P. lilacinum*, *Fusarium* sp., *Aspergillus nidulans* and *Scopuloriopsis* sp., reduced root knot nematode populations significantly. Field evaluation of the promising isolates clearly showed the supremacy of *P. chlamydosporia* in suppressing root knot nematodes in ginger (Eapen *et al.*, 2008). The combined application of *T. harzianum* + *P. fluorescens* + *B. subtilis* resulted in minimum population of root knot nematodes under field conditions (Dohroo and Gupta, 2014). Although none of these organisms is registered presently for use on ginger, they are potential tools for nematode management that may become available in the near future.

Radopholus similis

Parasitism of ginger by the burrowing nematode *R. similis* was first reported by Hart (1956) in Florida, USA. Later, Butler and Vilsoni (1975) and Turaganivalu *et al.* (2013) reported heavy infestation of ginger by *R. similis* in Fiji and its further spread through infested seed rhizomes. The occurrence of *R. similis* along with *M. incognita*, *Pratylenchus* sp. and *Helicotylenchus* sp. has also been reported from roots of ginger in India (Charles, 1978; Charles and Kurian, 1982).

Symptoms of damage

Infected plants usually exhibit stunting, reduced vigour and poor tillering. The topmost leaves become chlorotic with scorched tips, leading to yellowing at the base of shoots and the lower leaf sheath. Eventually, older leaves in the affected plants turn yellow, shoots eventually collapse, and discoloration extends further into the rhizome, leading to the total destruction of rhizomes (Turaganivalu *et al.*, 2013). Incipient infections of the rhizomes are evidenced by small, shallow, sunken, water-soaked lesions (Vilsoni

et al., 1976; Sundararaju *et al.*, 1979a). The nematodes migrate intracellularly through tissues, producing large infection channels or galleries within the rhizomes.

Means of dissemination

R. similis infestation of ginger fields in Fiji appears to have originated through bananas, as the areas once used for banana cultivation have been used for growing ginger (Vilsoni *et al.*, 1976). The coconut isolate of *R. similis* in Kerala (India) also reproduces well on ginger (Koshy and Sosamma, 1975, 1977). The perpetuation and dissemination of the nematode is through infested seed rhizomes used for planting.

Economic importance and population damage threshold levels

In Fiji, *R. similis* has been reported from more than 50% of the total area, with a rate of infection ranging from 10 to 50%, resulting in yield reductions of about 40%. An initial inoculum level of 10,000 nematodes/plant has been reported to cause 74% reduction in rhizome weight, and an initial inoculum level of 10 nematodes/plant reduced shoot weight, root weight and rhizome weight by 43, 56 and 40%, respectively, in a pot experiment (Sundararaju *et al.*, 1979c).

Management measures

Few studies have been done on the control of *R. similis* on ginger. Hot water treatment (51°C for 10 min) has long been recommended as a means of eliminating nematodes from ginger planting material (Colbran, 1969; Turaganivalu *et al.*, 2013). Crop rotation with taro and cassava and applying large quantities of poultry manure as fertilizer reduced populations of *R. similis* to very low levels (Turaganivalu *et al.*, 2013).

Pratylenchus

Several species of *Pratylenchus*, namely *Pratylenchus brachyurus*, *P. coffeae*, *Pratylenchus indicus*, *Pratylenchus pratensis* and *Pratylenchus zeae*, are reported on ginger (Charles, 1978; Das and Das, 1986; Kaur *et al.*, 1989; Kaur and Sharma, 1990) (Fig. 22.4).



Fig. 22.4. Ginger rhizomes showing necrosis and dry rot symptoms caused by infection with *Pratylenchus* sp. (Photograph courtesy of S.J. Eapen.)

Economic importance and symptoms

P. coffeae is reported to cause 'ginger yellows' disease, prevalent in Himachal Pradesh, India (Kaur and Sharma, 1990). The nematode is highly pathogenic to 15-day-old ginger seedlings, even at very low initial inoculum levels (Kaur, 1987). Nematode infestation caused yellowing of leaves and dry rot symptoms on rhizomes. Dark brown necrotic lesions were observed within the infected rhizomes (Kaur and Sharma, 1990).

Turmeric

Turmeric, *Curcuma longa* L., is best known as a condiment, although the plant has uses in the social and religious lives of people in South-east Asia, its probable origin. Commercial turmeric is the processed rhizome of *C. longa*. It is grown mostly in India, and to a small extent in China, Indonesia, Peru and Jamaica. In India, the total area under cultivation during 2014/15 was 184,410 ha, with a production figure of 830,350 t (DASD, 2016). It is cultivated as either a monocrop or an intercrop in many farming systems.

It is indispensable in the preparation of curry powder, and is an important source of natural yellow dye. It is also used as a colouring additive in the drug, confectionery and food industries. The rhizomes of *Curcuma aromatica*, a

close relative of *Curcuma longa*, are also a source of turmeric.

Nematodes on Turmeric

A number of species of plant parasitic nematodes have been reported in association with turmeric (Nirula and Kumar, 1963; Sundararaju *et al.*, 1979b; Chen *et al.*, 1986; Dasgupta and Rama, 1987; Gunasekharan *et al.*, 1987; Rama, 1987; Routaray *et al.*, 1987b; Bai *et al.*, 1995), of which *Meloidogyne* spp., *R. similis* and *P. coffeae* are of economic importance. *R. reniformis* and *M. incognita* were the most predominant and frequently recorded nematode species in the Chittor and Cuddapah districts of Andhra Pradesh (Mani and Prakash, 1992) and in Bihar (Haidar *et al.*, 1995) in India. *R. reniformis* was reported to be more harmful to turmeric than *M. incognita*, and caused a significantly greater reduction in plant growth (Haidar *et al.*, 1998a).

Meloidogyne

Two species of root knot nematodes, *M. incognita* and *M. javanica*, have been reported on turmeric, but most investigations have been concerned with *M. incognita*. Turmeric plants infested with *M. incognita* have large root galls, stunted growth, yellowing, marginal and tip drying of leaves, and reduced tillering with galling and rotting of roots. In the field, high densities of *M. incognita* cause yellowing and severe stunting and wilting in large patches. Plants die prematurely, leaving a poor crop stand at harvest. Infested rhizomes tend to lose their bright yellow colour (Mani *et al.*, 1987). Levels of protein, carbohydrate, chlorophyll a and b and curcumin were lower in plants infested with *M. incognita* (Poornima and Sivagami, 1998a). *M. incognita* completed its life cycle in turmeric within 30 days at 25–35°C and an adult female produced, on an average, 205 eggs (Mohanta and Swain, 2014).

The highest nematode multiplication and gall index were seen in peat soils (Poornima and Sivagami, 1998b). The population density of *M. incognita* increased with crop age and decreased with crop senescence (Poornima and Sivagami, 1999).

Economic importance and population damage threshold levels

One hundred juveniles of *M. incognita* caused significant reduction in the growth of turmeric plants (Haidar *et al.*, 1998a). A significant reduction in the growth and yield of turmeric were noticed in plants inoculated with more than 1000 root knot nematode juveniles/plant (Sudha *et al.*, 1989). When four cultivars of turmeric were tested against *M. incognita*, a maximum reduction of 18% fresh rhizome weight was observed in var. Suvarna at 2 juveniles/g of soil (National Research Centre for Spices, 1993). Poornima and Sivagami (1998a) reported that an initial inoculum level of more than 5000 *M. incognita* juveniles/plant was highly pathogenic to turmeric. By applying carbofuran at 3 kg a.i./ha, 3 weeks after planting, avoidable yield losses to the extent of 33.61 and 26.30% were observed in turmeric and ginger, respectively (Ray *et al.*, 1995). Avoidable yield loss under field conditions was 45.3% due to *M. incognita*, but was only 33.3% in a mixed infestation of *M. incognita* and *R. reniformis* (Bai *et al.*, 1995).

Management measures

RESISTANCE AND TOLERANCE: the cultivars and breeding lines 5379-1-2, 5363-6-3, Kodur, Cheyapuspa 5335-1-7, 5335-27, Ca-17/1, Cli-124/6, Cli-339, Armoor, Duggirala, Guntur-1, Guntur-9, Rajampet, Sugandham and Appalapadu have been reported as resistant to *M. incognita* (Gunasekharan *et al.*, 1987; Mani *et al.*, 1987). The species *Curcuma zedoaria* is more resistant to *M. incognita* than *C. domestica* in China (Chen *et al.*, 1986). In Andhra Pradesh, India, the high-yielding cultivars such as PCT8, PCT10, Suguna and Sudarshana were free from root knot nematode infestation (Rao *et al.*, 1994). Recently, Mohanta *et al.* (2015) screened 70 cultivars of turmeric and identified that cultivars Dugirala, PTS-31, Ansitapani, PTS-42 and PTS-47 were resistant to root knot nematodes, while 361 Gorakhpur, 328 Sugandham and PTS-21 were rated as moderately resistant. In India, out of 253 accessions screened against *M. incognita*, seven (Accs. 35, 48, 79, 130, 142, 146 and 200) were resistant. Field trials using these accessions indicated that in terms of yield,

one resistant accession (Acc. 79) and one moderately resistant accession (Acc. 48) performed significantly higher compared to the released cultivar IISR Prathibha (Prasath *et al.*, 2016).

PHYSICAL: immersing turmeric rhizomes in hot water at 55°C for 10 min or 45°C for 50 min can kill *M. incognita* inside rhizomes (Chen *et al.*, 1986). Soil solarization could be used for establishing nematode-free multiplication plots, but is unlikely to be economic for large-scale field use (Mohanta and Swain, 2013).

ORGANIC AMENDMENTS: adding poultry manure reduced root galling due to root knot nematode infection and increased the growth and yield parameters, and can be incorporated into integrated nematode management programmes (Udo and Ugwuoke, 2010). The incorporation of sesame oil cake (0.75 t/ha) at the time of soil solarization reduced the root knot nematode population and increased plant growth parameters (Mohanta and Swain, 2013).

CHEMICAL: although a few nematicides have been reported to control root knot nematodes of turmeric (Patel *et al.*, 1982; Gunasekharan *et al.*, 1987; Mani *et al.*, 1987; Haidar *et al.*, 1998b), farmers prefer non-chemical means to control nematodes.

BIOLOGICAL: the biocontrol agents *P. chlamydosporia*, *P. lilacinum*, *Fusarium* sp., *Aspergillus* sp. and *Scopuloriopsis* sp. controlled root knot nematodes in field trials but have not been tested in growers' fields (Ramana *et al.*, 2002; Eapen *et al.*, 2008).

Radopholus similis

Symptoms of damage

The roots of turmeric damaged by *R. similis* become rotted, and most of these decayed roots retain only the epidermis devoid of cortex and stelar portions. The infested plants show a tendency to age and dry faster than healthy plants. Infested rhizomes are of a yolk-yellow colour compared with the golden-yellow colour of healthy rhizomes, and have shallow, water-soaked,

brownish areas on the surface. The scale leaves harbour *R. similis* (Sosamma *et al.*, 1979).

Survival and means of dissemination

The nematodes are disseminated through infested planting material. Populations of *R. similis* from coconut are known to infest turmeric (Koshy and Sosamma, 1975), and the use of turmeric as an intercrop in *R. similis*-infested coconut- and arecanut-based farming systems should be avoided.

Economic importance and population damage threshold levels

Pathogenicity studies show that an initial inoculum level of ten nematodes per plant can cause a reduction of 35% of the rhizome weight after 4 months and a 46% reduction at the end of the season (8 months). With 100,000 nematodes, the extent of reduction in rhizome weight is 65 and 76% after 4 and 8 months, respectively (Sosamma *et al.*, 1979).

Management measures

Control has not been studied under field conditions. However, the use of clean, nematode-free rhizomes for planting should be the first step in developing an integrated management system for the burrowing nematode on turmeric.

Pratylenchus coffeae

P. coffeae has been reported to be associated with the discoloration and rotting of mature rhizomes of 'wild turmeric', *C. aromatica*. In advanced stages of infection, the rhizomes become deep red to dark brown in colour, less turgid, and wrinkled with dry rot symptoms. The fingers are affected more severely than the mother rhizomes. Internally, the affected rhizomes show dark brown necrotic lesions (Sarma *et al.*, 1974).

Future prospects

Turmeric has received very little input in terms of nematological research, although *M. incognita*,

M. javanica, *R. similis* and *P. coffeae* are known to damage the crop. Detailed investigations, including surveys and pathogenicity experiments, and control through resistant/tolerant cultivars and cultural, chemical and biological methods, are warranted.

Other Spices

Although a number of spice crops including tree spices and seed spices are cultivated over large areas in the tropics and subtropics, there is very little information available on the damage and yield loss caused by plant parasitic nematodes on some of these crops. This is not to say that nematode problems do not exist on these crops, but only that there has been a lack of nematological investigations. The plant parasitic nematodes that have been reported in association with these crops in surveys and host range studies are given in Table 22.1. Nematodes have been found in: clove (Ghesquiere, 1921; Goodey *et al.*, 1965; Sharma and Loof, 1974; Bridge, 1978; Sundararaju *et al.*, 1979b); nutmeg (Goffart, 1953; Goodey *et al.*, 1965; Kumar *et al.*, 1971; Sundararaju *et al.*, 1979b; Chawla and Samathanam, 1980); cinnamon (Goffart, 1953; Goodey *et al.*, 1965; Sundararaju *et al.*, 1979b; Chawla and Samathanam, 1980; Dasgupta and Rama, 1987; Rama, 1987); cumin (Swarup *et al.*, 1967; Verma and Prasad, 1969; Shah and Raju, 1977; Shah and Patel, 1979; Patel *et al.*, 1986; Midha and Trivedi, 1991); fennel (Midha and Trivedi, 1991); fenugreek (Chandwani and Reddy, 1967; Krishnamurthy and Elias, 1967; Khan and Khan, 1969, 1973; Mathur *et al.*, 1969; Rashid *et al.*, 1973; Khan, 1975); coriander (Chandwani and Reddy, 1967; Krishnamurthy and Elias, 1967; Sen and Dasgupta, 1977; Das and Sultana, 1979; Midha and Trivedi, 1991) and vanilla (Orton Williams, 1980; Stier, 1984, in Bridge, 1988). All these spices are hosts of *Meloidogyne* spp. The roots of cumin can also be severely galled by *M. incognita* and *M. javanica* (Patel *et al.*, 1986). *P. brachyurus* is reported to be a parasite of vanilla in the Pacific island of Tonga, causing reduced growth of vines (Stier, 1984, in Bridge, 1988).

Related Crops

Betel Vine

The betel vine *P. betle* is a perennial, dioecious, semi-woody creeper, probably native of Malaysia. Its leaves are used for chewing, the extraction of essential oils such as methyl eugenol and in traditional herbal (ayurvedic) medicines and religious ceremonies. It is grown throughout Asia and also in Africa, the Philippines, Indonesia and the Pacific islands. The area under betel vine cultivation in India is about 30,000 ha, with an annual turnover of 7000 million Indian rupees. The yield varies from 7.5 to 22.5 million leaves/ha/year (Shenoy, 1985).

Its cultivation is labour-intensive and requires heavy investment. Betel vine is propagated by cuttings of 3–5 nodes from 2-year-old vines. It is trailed on coconut, arecanut or other straight-stemmed plants such as *Sesbania grandiflora*, *Moringa oleifera* and *Erythrina variegata*. Non-living standards such as bamboo, wooden poles or granite stone supports are also used. The crop is usually heavily manured with farmyard manure, oil cakes, fish manure, sheep manure, etc.

Nematodes on Betel Vine

Numerous plant parasitic nematodes have been reported associated with the betel vine in India and elsewhere (Timm, 1965; Reddy, 1978; Ganguly and Khan, 1983; Sivakumar and Marimuthu, 1984, 1985; Jagdale *et al.*, 1986a,b; Acharya *et al.*, 1988; Ganguly, 1988; Ganguly and Khan, 1990; Nema, 1997). The yield losses owing to nematode diseases were expected to be 4–41% (Kumar *et al.*, 2016), while root knot nematode *M. incognita* alone has been reported to cause 30–38% yield losses (Jonathan *et al.*, 1990).

Meloidogyne incognita

M. incognita has been reported to be associated with betel vine decline from all areas in India (Dhande and Sulaiman, 1961; Venkata Rao *et al.*, 1973; Mammen, 1974; Sivakumar and Marimuthu, 1984; Jagdale *et al.*, 1986a).

Symptoms of damage

Infested plants exhibit poor growth, yellowing of leaves, reduced vigour and wilting, with heavy galling and rotting of roots (Jagdale *et al.*, 1986a). Thinly spread foliage with small leaves, yellowing and premature shedding of leaves and stunting were recorded in root knot nematode-infested vines (Acharya and Padhi, 1987a).

Disease complexes

Association of *M. incognita* with severe wilt symptoms of betel vine has been reported from India (Mammen, 1974). *M. incognita* predisposed betel vine to root rot caused by *Phytophthora palmivora* (Sivakumar *et al.*, 1987; Marimuthu, 1991; Jonathan *et al.*, 1996) and *P. capsici* (Sitaramaiah and Devi, 1994). Pathogenic association of *M. incognita* with *Sclerotium rolfsii* and *Xanthomonas beticola* has also been reported (Acharya *et al.*, 1987; Sitaramaiah and Devi, 1990). A disease complex involving *M. incognita* and *Colletotrichum* sp. has also been reported in betel vine (Ray *et al.*, 1993).

Economic importance and population damage threshold levels

The root knot nematode is damaging to betel vine at an initial inoculum level of 100 juveniles/plant (Jagdale *et al.*, 1985a). The leaf yield of untreated plants was reduced by 38% over carbofuran-treated plants (Jonathan *et al.*, 1990). Avoidable yield losses under field conditions in Assam were estimated at 17.95% in terms of the number of leaves and at 29.06% in terms of the fresh weight of leaves (Hazarika *et al.*, 1999b).

Management measures

CULTURAL: a crop rotation of betel vine–rice–banana–rice is helpful in reducing *M. incognita*, *Helicotylenchus* sp. and *R. reniformis* populations on betel vine raised in rice fields (Sivakumar and Marimuthu, 1986a; Sivakumar *et al.*, 1987). Considerable reduction in the nematode populations in soil and the number of galls on roots was reported after the application of 50–75 kg of K_2O /ha (Jagdale *et al.*, 1985e; Rabindran *et al.*, 1987). Likewise, growing *Tagetes erecta* in the basins of betel vines significantly reduced root knot nematodes (Medhane *et al.*, 1985). Nematode-

susceptible standards such as *S. grandiflora* and *Sesbania sesban* should not be used for trailing the vines (Rao *et al.*, 1991). In another study, the application of decaffeinated tea waste and mustard oil cake at 1 kg/plant reduced nematode populations and returned significantly higher yields (Hazarika *et al.*, 1999a).

The application of neem oil cake at 1 t/ha and sawdust at 2 t/ha was reported to reduce nematode populations and the number of galls and increased significantly the number of leaves harvested (Jagdale *et al.*, 1985b,c; Acharya and Padhi, 1988a). Similarly, a significant reduction (60%) in the nematode population was observed in beds amended with chopped and shade-dried leaves of *Calotropis gigantea* at 2.5 t/ha, followed by neem oil cake and poultry manure at 44.4 and 40.9%, respectively. Beds amended with *C. gigantea* leaves yielded 14.2 kg of 4840 leaves and with neem oil cake 12.1 kg of 4220 leaves. Soil amendment with sawdust at 2 t/ha + NPK and neem oil cake at 2 t/ha was effective in reducing nematode numbers and increasing yields (Sivakumar and Marimuthu, 1986b; Rana *et al.*, 1991; Murthy and Rao, 1992, 1994). In another study, the highest reduction in nematode population (43%) was obtained with the application of neem seed cake at 0.5 t/ha together with carbofuran at 0.75 kg a.i./ha (Nema, 2001a).

RESISTANCE AND TOLERANCE: the cv. Karpoori is highly susceptible, whereas the cv. Kuljedu has the lowest root knot index and number of egg masses per plant (Jagdale *et al.*, 1985d; Sivakumar *et al.*, 1987). In addition, the cvs. Kakair, Bangla, Karapaku, Gachipan, Aswani pan and Berhampuri have been reported to be tolerant to root knot (Anon., 1987). The cultivar Berhampuri has also been reported by other workers to be less susceptible to this nematode (Acharya and Padhi, 1988b). Another cv., Bangla Budagar, was found to be moderately resistant to *M. incognita* (Nema, 2001a).

PHYSICAL: solarization by mulching the land with 100 gauge black and white polythene for 15 days before planting was found to reduce plant parasitic nematode populations in India (Sivakumar and Marimuthu, 1987; Rao *et al.*, 1996).

BIOLOGICAL: the root knot nematode problem in betel vine has been controlled through the

application of the biocontrol fungus *P. lilacinum* (Jonathan *et al.*, 1995, 2000; Hazarika *et al.*, 1998, 2000; Nakat *et al.*, 1998; Pathak and Saikia, 1999; Bhatt *et al.*, 2002b). The application of *Trichoderma viride* multiplied on linseed oil cake was also found to be highly effective in reducing root knot incidence in betel vine (Bhatt *et al.*, 2002a).

CHEMICAL: field application of non-fumigant nematicide has been shown to reduce root knot nematode populations (Jagdale *et al.*, 1984), in some cases by between 71 and 55%, resulting in increased yields (Sivakumar *et al.*, 1987). Application in the furrow on either side of the rows significantly reduced *M. incognita* populations in soil and galling of the roots (Dethe and Pawar, 1987). However, the use of systemic nematicides is generally not recommended for betel vine, as the leaves are picked continuously and consumed directly without any processing. Because of problems with nematicide residues in leaves (Pattnaik *et al.*, 1989; Rao *et al.*, 1993; Mahapatra and Awasthi, 1994), root knot nematode infestations on betel vine must be solved by integrated nematode management, by following the points outlined below:

- Crop rotation wherever possible.
- The use of resistant/tolerant cultivars.
- The use of non-living standards or nematode-resistant live standards for supports.
- Solarization by mulching with 100-gauge clear polythene before planting.
- The application of organic amendments such as neem or *Calotropis* leaves and sawdust at 2 t/ha.
- The supply of nitrogen through neem oil cake at 2 t/ha.

Radopholus similis

The burrowing nematode *R. similis* has been reported to cause 'yellows' or 'slow wilt' disease of betel vine in India. The symptoms produced on betel vine are akin to the symptoms caused by *R. similis* on black pepper vines (Koshy and Sosamma, 1975; Sundararaju and Suja, 1986; Eapen *et al.*, 1987). The integrated management schedules suggested for the control of nematodes on black pepper, other than the application

of nematicides, can be largely adopted with modification to suit local conditions for controlling *R. similis* on betel vine. The inoculation of plants with *P. lilacinum* 25 days prior to *R. similis* was effective in reducing plant damage (Sosamma *et al.*, 1994).

Rotylenchulus reniformis

Acharya and Padhi (1987b) and Bhatt *et al.* (2002b) found *R. reniformis* to be pathogenic to betel vine. At inoculum levels of 1000 and 20,000 nematodes/cutting, a reduction by 20 and 60%, respectively, was observed in the number of leaves. Ganguly (1988) reported *R. reniformis* as the dominant species found to be associated with five cultivars of betel vine in Maharashtra. *R. reniformis* interacted synergistically with *P. palmivora* to increase vine mortality (Jonathan *et al.*, 1997).

Kava

Kava or Yaqona (*P. methysticum*) provides a popular narcotic drink for the peoples of the Pacific islands. The drink is made from the thick roots of this bushy shrub.

Nematodes of Kava

Root knot nematodes, *Meloidogyne* spp., have been found to be associated with a serious disease of kava, and the nematodes alone can decrease the growth of plants greatly in Fiji and Tonga (Stier, 1984, in Bridge, 1988). Fliege and Sikora (1981) reported *M. incognita* causing severe root galling of *P. methysticum* in Western Samoa.

Other potentially damaging parasitic nematodes that have been found with kava include *R. reniformis*, *P. coffeae* and *R. similis* (Kirby *et al.*, 1980; Orton Williams, 1980). None of these have as yet been shown to cause economic damage to the crop. Further investigations are necessary to determine the economic importance of nematodes, particularly *Meloidogyne* spp., and their means of control.

Medicinal Plants

Plant parasitic nematodes are associated with almost all medicinal plants studied to date, and often cause significant damage. However, the magnitude of crop damage has only been established for a few of these nematode–plant interactions (Pandey *et al.*, 2003). Three species of plant parasitic nematodes are considered of economic importance on medicinal plants: the root knot nematodes (*M. incognita* and *M. javanica*), the lesion nematode (*Pratylenchus thornei*) and the stunt nematode (*Tylenchorhynchus vulgaris*). Root knot nematodes are the most important nematode parasites limiting production, with infestations reported on menthol mint, henbanes, basil, opium poppy, ashwagandha, sarpgandha, coleus, kinghao, brahmi and musli (Pandey, 1998b, 2003), as well as on jaborandi (*Pilocarpus microphyllus*) (R.A. Sikora, Germany, 2004, personal communication).

Henbanes

Henbanes (*Hyoscyamus muticus*, *Hyoscyamus niger* and *Hyoscyamus albus*) are important tropane alkaloid-bearing plants belonging to the family Solanaceae and one of the chief sources of tropane alkaloids (hyoscyine, scopolamine, hyoscyamine, atropine, etc.) obtained from the dried leaves and other plant parts.

Meloidogyne

Although many plant parasitic nematodes have been reported to be associated with different species of henbane, only root knot nematodes cause serious damage to the crop. Henbanes have been reported to be heavily infested with *M. incognita* and *M. javanica* in India (Pandey, 1990).

Symptoms of damage

Root knot-infested plants of *H. muticus*, *H. niger* and *H. albus* show chlorosis and stunting, and the plants have fewer and smaller leaves and flowers. The roots of infested plants are often severely galled. A pre-plant density of 3–4 juveniles/g of soil caused significant damage to the crop (Haseeb and Pandey, 1989; Pandey, 1990).

Management measures

Crops resistant to root knot should be used in rotation with henbanes to reduce pre-plant nematode densities in the soil. No henbane species screened have been proved to be resistant to the nematodes (Pandey, 1998b).

The nematicides carbofuran at 2 kg a.i./ha of soil and monocrotophos at 0.1% in solution have been used to reduce root knot nematode damage in henbane. Monocrotophos was used to soak seeds prior to planting, and carbofuran was applied to the soil prior to sowing of the crop. The combined treatment effectively reduced root knot infestations (Pandey, 2000a).

When *H. niger* was inoculated with the plant health-promoting rhizobacteria *P. fluorescens* or with one of three species of arbuscular mycorrhizal fungi (*Glomus aggregatum*, *G. mosseae* or *G. fasciculatum*), *M. incognita* densities were reduced and plant biomass increased. The use of a combination of antagonists proved to be the most effective in managing the disease (Pandey, 1997; Pandey *et al.*, 1999, 2000b, 2000c; Mishra *et al.*, 2012).

In pot tests, essential oils of *Cymbopogon martinii*, *Cymbopogon wintrianus*, *Ocimum basilicum* and *Mentha arvensis* were effective in reducing *M. incognita* populations and improved the growth of *H. niger*; the oil from *C. martinii* at 2 ml/plant was found to be most effective (Pandey *et al.*, 2000a).

Ashwagandha (*Withania somnifera*)

Withania somnifera is a valuable medicinal plant with strong antioxidant, anti-inflammatory, anticancerous, anti-stress, anti-aging, immunomodulatory properties and its therapeutic actions are due to the presence of steroidal lactones and acyl sterol glycoside in the plant. In Asian countries, the roots of *W. somnifera* are used to treat hiccups, coughing, dropsy, rheumatism and as a sedative.

Nematodes of Ashwagandha

During a survey to collect new germplasm, almost all *W. somnifera* plants sampled were found to be

galled by *M. incognita* race 2 (Pandey and Kalra, 2003). Infected plants were chlorotic, stunted, less branched and with fewer and smaller leaves. The roots of such plants were severely galled. When the stem touched the soil, it was also found to be infested with the nematode (Fig. 22.5).

Management measures

Amendments from the neem plant (*A. indica*), marc from *Artemisia annua* as well as distillates from *M. arvensis* and *Bergera koengii* (syn. *Murraya koenigii*) were found to reduce *M. incognita* densities on *W. somnifera* (Pandey *et al.*, 2003). In glasshouse and field experiments, various microbes, namely *T. harzianum*, *T. viride*, *P. lilacinum*, *Arthrobotrys oligospora*, *G. aggregatum*, *Glomus intraradices*, *B. megaterium*, *Bacillus tequilensis* and *P. fluorescens*, in combination with organic materials were found to be effective in reducing the root knot nematode population, galling and enhanced plant secondary metabolites in *W. somnifera* (Pandey *et al.*, 2003; Sharma and Pandey, 2009; Pandey *et al.*, 2011a; Saikia *et al.*, 2013; Gupta *et al.*, 2016; Tiwari *et al.*, 2016).



Fig. 22.5. Large root galls on *Withania somnifera* infested with *Meloidogyne* sp. (Photograph courtesy of R. Pandey.)

Brahmi (*Bacopa monnieri*)

Bacopa monnieri, commonly known as brahmi, is the chief source of baccoside A and B, which are used extensively in the formulation of medicines used to treat asthma, epilepsy, mental disorder and as a memory enhancer.

Nematodes of Brahmi

Although a number of plant parasitic nematodes are associated with brahmi, only the root knot nematode *M. incognita* causes serious damage to the crop. The nematode causes stunting and leaf chlorosis (Fig. 22.6). In greenhouse trials, a negative correlation between increasing population levels of *M. incognita* and plant growth of *B. monnieri* was demonstrated (Pandey *et al.*, 2003).

Management measures

The amendments and distillates combined with the fungal antagonists discussed above in



Fig. 22.6. *Bacopa monnieri* plant infested with *Meloidogyne* sp. (Photograph courtesy of R. Pandey.)

the section on control in ashwagandha were also successful in reducing root knot densities and enhancing the growth and yield of *B. monnieri* (Pandey *et al.*, 2003; Gupta *et al.*, 2015).

Chlorophytum borivillianum

Chlorophytum borivillianum, commonly known as safed musli, is an important medicinal plant belonging to the family Liliaceae. This plant is distributed widely throughout India. The presence of saponins and alkaloids in this plant is of high medicinal importance. Progressive farmers in India are cultivating the crop for both the local and the international herbal industry. The tuberous root is sold in the market for medicinal use and is also saved for planting the next crop. There are several species of *Chlorophytum* grown in India.

Nematodes of *Chlorophytum borivillianum*

Several nematode species are associated with *C. borivillianum*, but the root knot nematode *M. incognita* poses a major threat to successful cultivation of this crop. The nematode parasitizes the fine root system and completes its life cycle within the plant tubers. The nematode therefore causes severe tuber loss when present in the cropping field (Pandey *et al.*, 2003). Plants in infested fields are stunted and have drooping leaves that dry over time.

Management measures

The utilization of *B. megaterium*, *P. fluorescens*, *G. intraradices* and *T. harzianum* CIMAP-RPN01, alone as well as in combinations, gave successful management of *M. incognita* and enhanced the growth/yield of *C. borivillianum* (Pandey and Saikia, 2014).

The following integrated approach to combat the root knot nematode problem has been suggested (Pandey *et al.*, 2003):

- Plant healthy tubers that are nematode free.

- Pre-treat the soil with carbofuran at 2.0–3.0 kg. a.i./ha before planting.
- Treat the tubers with a mixture of biological control agents.

Mint (*Mentha* spp.)

Among the different medicinal and aromatic plants, mints are of major pharmaceutical importance due to their many-fold uses. Farmers in the tropics and subtropics can grow mint as a cash crop whenever it fits into a cropping system. The crop generates significant local employment and earns foreign exchange. The main types of mints cultivated commercially in tropical and subtropical countries are: menthol mint (*M. arvensis*), peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), scotch spearmint (*Mentha cardiaca*), bergamot mint (*Mentha citrata*) and garden mint (*Mentha viridis*).

Nematodes of Mints

Nematodes have been identified as major pests of several mint species. The important nematodes reducing yield are species of *Meloidogyne*, *Pratylenchus* and *Tylenchorhynchus* (Pandey *et al.*, 2010). Several other plant parasitic nematodes are associated with these mint species, but are still of unknown economic importance (Pandey, 1999; Pandey *et al.*, 2011b).

Meloidogyne

M. incognita particularly, and also *M. javanica*, are important yield-limiting factors in menthol mint wherever it is cultivated.

Symptoms of damage

Root knot-infested mint plants are stunted and chlorotic, with damage occurring in typical oval patches throughout the field. Root knot-infested suckers or roots bear galls of various sizes (Fig. 22.7), and egg masses are clearly visible on the root system under the microscope.

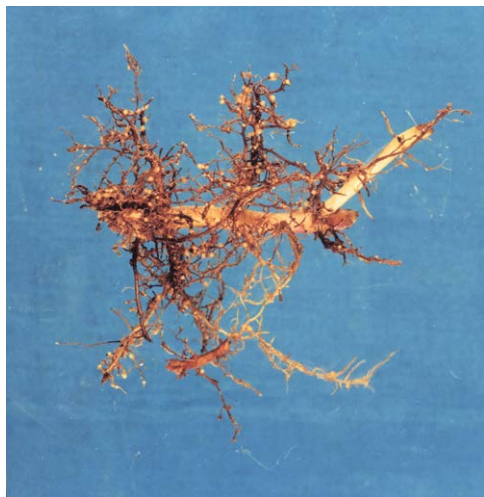


Fig. 22.7. Suckers of menthol mint, *Mentha arvensis*, infested with *Meloidogyne* sp. (Photograph courtesy of R. Pandey.)

Biology

The life cycle of *M. incognita* in menthol mint is completed within 28–30 days, with up to four generations developing per season under favourable conditions. Race 2 of *M. incognita* is predominant in the Lucknow area of Uttar Pradesh, India (Pandey *et al.*, 1992).

Survival and dissemination

Because *Meloidogyne* juveniles and eggs survive inside the storage roots and suckers, the nematode is often disseminated in planting material if care is not taken to avoid contamination. Soil adhering to the suckers is also a good means of spread. The presence of alternative weed hosts in a field is important in maintaining root knot nematode inoculum between crops.

Environmental factors

Meloidogyne multiplies well in the sandy soils generally used to cultivate menthol mint; therefore, damage caused by root knot nematodes in these regions is often severe (Pandey *et al.*, 1992). Menthol mint is also transplanted in January, when temperatures are optimum for nematode infection and development, resulting in 3–4 generations per growing season and high levels of damage (Pandey, 1989).

Economic importance

Meloidogyne species reduce plant growth and oil yield significantly. In addition, *M. incognita* multiplies on all species of *Mentha*, as well as on all cultivars (Pandey, 1989; Pandey *et al.*, 1992). Strong reductions in plant growth as well as in the rate of photosynthesis were found to be correlated directly with initial inoculum densities. *M. incognita* and *M. javanica* caused a 25–30% reduction in oil yield in menthol mint; the quality of the mint oil is also affected adversely by nematode infection (Pandey, 1998a, 2003).

Management of nematodes in menthol mint

Root knot infection was reduced when plants were pre-inoculated with different combinations of arbuscular mycorrhizal fungi (Pandey *et al.*, 1997, 1998). The symbionts reduced nematode infection and improved oil yield.

Successful control of root knot was also achieved with the application of carbofuran at 1.5 kg a.i./ha or with neem cake at 500 kg/ha (Pandey, 2000b, 2003).

The management of *M. incognita* using biological control agents, organic matter and the integration of both was studied by Pandey (1993, 1995, 1998a, 2000a). The arbuscular mycorrhizal fungi (*G. aggregatum*, *G. mosseae* and *G. fasciculatum*), the antagonist (*T. harzianum*) and the oil seed cakes from mustard (*Brassica campestris*) and from neem (*A. indica*), along with the nematicide carbofuran, were effective in increasing the yield of menthol mint and in reducing root knot densities. Maximum reduction in the nematode population was recorded in the neem cake-treated soil, followed by mustard cake, carbofuran and then the biological control agents. Significantly higher levels of yield were obtained in the following order: neem, mustard, *T. harzianum*, *G. aggregatum* and then carbofuran. The use of vermicompost and different distillation waste products was also found to enhance the growth and yield of different mint species and reduce nematode populations significantly (Pandey *et al.*, 2003). Pre-inoculation of mutualistic *T. harzianum* along with *G. intraradices*, *P. fluorescens* and *B. megaterium* was found to be highly beneficial for enhancing menthol mint yield and reducing *M. incognita* infestation (Pandey *et al.*, 2011b).

Table 22.2. Utilizing crop rotation to increase yield as well as minimizing the root knot nematode populations in menthol mint. (From Pandey, 2003.)

Crop rotation	Net benefit (rupees)	Root knot index in menthol mint
1 Maize–potato–menthol mint	83,000	+
2 Paddy–potato–menthol mint	80,000	+
3 Paddy–pea–menthol mint	74,000	+++
4 Maize–mustard–menthol mint	76,000	++
5 Pigeonpea–menthol mint	72,000	++
6 Paddy–menthol mint	75,000	++
7 Paddy–wheat–menthol mint	68,000	++

Note: + = mild; ++ = moderate; +++ = severe infestations.

Resistance

Germplasms available in the gene bank at the CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP) in Lucknow, India, were screened for resistance to *M. incognita* (Pandey and Patra, 2001). Moderate to high degrees of resistance were observed on SS-1-4, SS-2-7, SS-15, SS-26, SS-36, *M. piperita* cv. Kukrail, *M. spicata* cv. Neera, *M. spicata* cv. Arka, *M. citrata* cv. Kiran, *Mentha gracilis* and *Mentha viridis*.

Non-host crops such as mustard and wheat have been shown to reduce root knot populations and increase the yield of menthol mint (Table 22.2). The Trichoderma technology and 'Early Mint Technology' developed at CSIR-CIMAP in Lucknow have also benefited crop health and yield greatly. The higher temperatures prevailing during late transplanting (April–July) adversely affect nematode population build-up and infection of the menthol mint crop (Pandey, 2003).

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23 Management Practices: An Overview of Integrated Nematode Management Technologies*

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Nematode management is not static, but changes as external factors alter grower needs, as nematode problems shift in importance and with the development of new methods of management. Management first moved from simple dependence on crop rotation with non-host crops to integrated pest management (IPM) that initially relied on a combination of crop rotation and chemical control. IPM then expanded toward more complex integrated crop management (ICM), with emphasis placed on the use of resistant cultivars. With the removal of many important nematicides from the market, there was a shift in IPM toward integrated management with emphasis on biologically based system management options. These changes in approaches have been reviewed in detail (Bridge, 1996; Sikora, 1992; Sikora *et al.*, 2005; Ciancio and Mukerji, 2008; Stirling, 2014).

Nematode management in modern commercial agriculture today is holistic and relies on the integration of traditional and modern technologies to reduce or prevent nematode infection and crop loss. Control, therefore, has progressed from the very early misconception that nematodes could be eradicated by rotation and treatment with nematicides, toward more sustainable approaches with the ultimate goal of cost-effective nematode management, higher yields and reduced

impact on the environment. The need for new forms of management has been generated by major changes in agricultural production, how the public views the production of their food and the negative impacts agriculture has on the environment.

A large number of overriding factors has stimulated the need for more research on nematode management, including:

- Extreme shortening of rotations;
- Expansion of protected cultivation of vegetables;
- Loss of effective non-fumigant and fumigant nematicides;
- Detection of virulent resistant-breaking populations;
- Globalization and increased nematode spread;
- Detection of new plant parasitic nematode species;
- Global warming favouring nematode multiplication.

These drivers of change have caused marked shifts in research and the development of new nematode management technologies, including:

- Remote sensing technologies and targeted nematicide application;

*A revision of the chapter by R.A. Sikora, J. Bridge and J.L. Starr in the second edition.

- Minimum tillage and activation of the antagonistic potential;
- Improved nematicide screening and new modes of action;
- Bioenhancement of planting material with biologicals;
- Targeted biological/chemical seed treatment;
- Advanced marker-orientated breeding programmes;
- Development of stringent quarantine regulations;
- Molecular diagnostic tools for monitoring and detection;
- Advanced methods of screening for resistance;
- New tools for biodiversity indexing including metagenomics.

All of these developments have stimulated new approaches for nematode management in the crops reviewed in this book. However, when nematode management is discussed in the tropics and subtropics, we often overlook the fact that the majority of the world's cultivated land is still farmed by small-scale farmers using traditional methods (Altieri, 1984; FAO, 2014; Vanlauwe *et al.*, 2014). On a global scale, nematodes often play an overlooked role in food security, especially in subsistence crops that are the main source of food for the poor. These food crops are grown by resource-limited small-holders having less than 2 ha of land. For these farmers, nematodes are still an unseen enemy. Research in nematology, directed at small family farms or subsistence agriculture, is urgently needed, because most integrated crop management practices used in modern agriculture are not available or affordable to these growers.

Nematode management, therefore, is often driven by whether the ultimate goal is food for survival (subsistence agriculture) or food for the marketplace (cash-crop commercial farming). Both systems are driven by resource availability, which varies from almost non-existent in subsistence agriculture to available but costly in market-driven commercial production. Management also varies greatly between the different farming systems: subsistence, resource-limited, conventional small-scale, modern extensive and high-intensity commercial production. Flexibility in designing nematode management options,

therefore, depends on a grower's needs (food or income generation), knowledge and resource availability.

The occurrence of a serious nematode problem is often one of the first indications that a farming system has become unsustainable (Page and Bridge, 1993; Sikora *et al.*, 2005). Truly indigenous nematodes are generally not a problem in traditional mixed-cropping systems where many different crops are planted randomly throughout a farm. Nematodes in these field situations become serious pests because of the unintended introduction of the pest or due to a change in the cropping system toward fewer crops and/or narrower rotations, often with good host plants. Conversely, the existence of nematode problems in modern commercial production is usually known and management tools that are effective are accessible.

In addition, there is an enormous spectrum of cropping patterns in tropical and subtropical agriculture that varies greatly over a 12-month period:

- Monoculture with a single perennial crop, e.g. citrus, banana;
- Rotation with two crops, e.g. cereal-legumes in India;
- Monoculture of a single crop planted three times sequentially, e.g. rice in Asia;
- Sequentially planted multiple-crop rotations, e.g. wheat-legumes-rice in Asia;
- Relay and sequentially planted vegetables Asia, the USA, southern Europe;
- Totally randomized mixed-cropping systems with multiple crops, Africa, Asia.

The intensity and complexity of cropping patterns used in the tropics includes combinations of alley, relay and sequential planting (Fig. 23.1). These cropping patterns are used extensively in Asian vegetable production, where up to 11 short-maturation, high-value horticultural crops are planted in a field over a 12-month period (see Chapter 10, this volume). Very often, a break crop of paddy rice is used to suppress soil-borne pest and disease populations between the planting of the susceptible crops. Similar patterns of production can be used in subtropical vegetable production, where up to five crops can be grown sequentially when nematodes are properly controlled (J.W. Noling, USA, 2017, personal communication). In Asia, a food-feed intercropping

Nematode management in the Mediterranean region

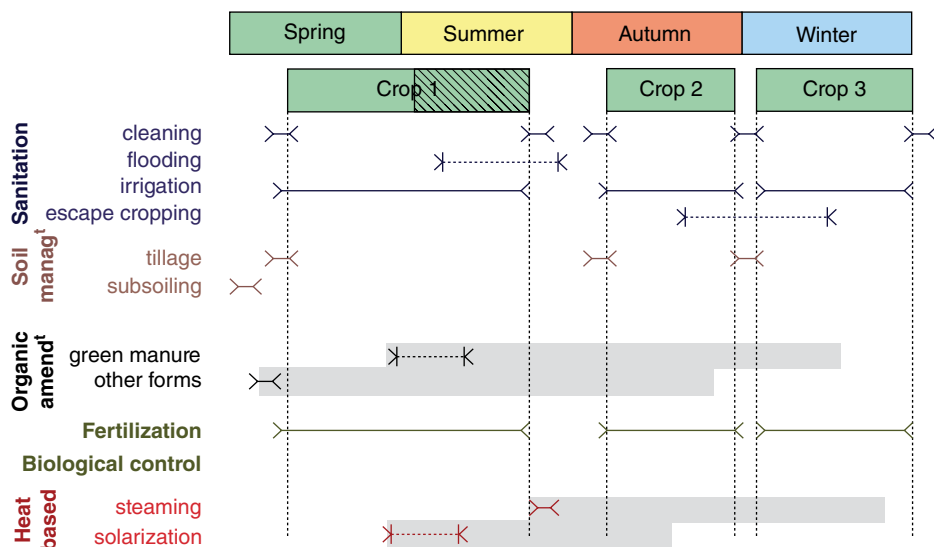


Fig. 23.1. Diversity of cropping patterns and the positioning of integrated nematode management tools used in crop sequences in the Mediterranean region. (From Collange *et al.*, 2011.)

system to support animal production in rice rotations has been proposed (Devendra, 1993).

In the mixed-cropping systems that typify the small family farms of many tropical countries, multilayer cropping is also practised. In these farms, perennial and annual crops are intercropped for reasons of support or shade in a highly randomized pattern that gives these farmers a variety of food but also a degree of insurance against crop loss due to biotic and abiotic factors (Fig. 23.2). In many cases, nematodes are present that attack multiple crops in the field and lead to severe yield reduction.

This chapter draws heavily on the personal experience of the authors and material presented in the chapters in this edition. The chapter is not to be seen as a thorough review of the literature. Excellent books and reviews have been devoted to different aspects of integrated nematode management and should be consulted for more in-depth information for structuring management programmes (Brown and Kerry, 1987; Barker *et al.*, 1998; Whitehead, 1998; Cook and Starr, 2006; Ciancio and Mukerji, 2008; Viaene *et al.*, 2013). Whatever management approach is taken, the ultimate goal is

a stable and/or higher yield, and in most cases, increased profit. The tools used in the management of nematodes can, and often, must be applied at different times in a cropping cycle or in a rotation sequence (Figs 23.1 and 23.2).

Because of the complexity of many tropical and subtropical cropping programmes, we decided to discuss each management tool according to its importance at specific states in the production of a single susceptible crop. A similar approach was taken by Collange *et al.* (2011).

In some cases, the technology used could be applied more than once in a single year, depending on the cropping pattern. The stages in production where specific management tools can be used are:

- Exclusion, quarantine and diagnosis;
- Inter-cycle management between susceptible crops;
- Pre-plant management immediately prior to planting;
- At-planting management;
- Established crop management;
- Postharvest management;
- Integrated nematode management strategies.

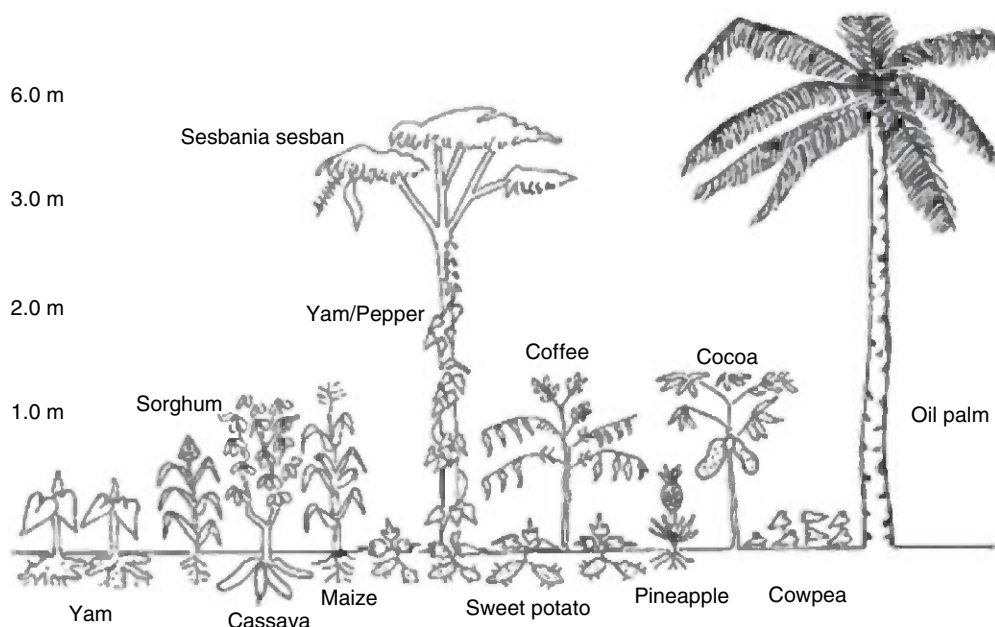


Fig. 23.2. Multilayer intercropping of annual and perennial food crops in the tropics. (From FAO, 2001.)

Exclusion, Quarantine and Diagnosis

Exclusion is the most effective and economical means of preventing nematode damage. Preventing the introduction of nematode pests from abroad, as well as within a country or region, has previously proved effective in limiting nematode spread and damage and should be continuously strengthened. The global market for agricultural products has promoted not only efficient long-distance movement of plants, fruit and grain but also the spread of important nematode pests around the world. Some examples of the introduction of economically important nematodes include: pine wilt nematode *Bursaphelenchus xylophilus* to Europe; soybean cyst nematode *Heterodera glycines* to Brazil; burrowing nematode *Radopholus similis* worldwide; red ring nematode *Bursaphelenchus cocophilis* to South America; and *Globodera* spp. to Europe. Nematodes detected in different parts of the world that could at some point be important to agriculture and that need to be considered by quarantine agencies on an individual country basis are listed in Table 23.1. The most economically important species of plant parasitic nematodes, regulated

by quarantine agencies worldwide, are listed in Table 23.2. Distribution maps, updated on a regular basis, that show the worldwide distribution of many economically important species are available from CABI (2016). These maps should be consulted for detailed information on the distribution of species important to quarantine agencies. The prevention of spread at a local or country level can lead to significant reductions in crop loss, as well as savings in production. Hockland *et al.* (2006) reviewed the main aspects important in developing legislation and other aspects related to phytosanitary initiatives for use in developing quarantine programmes aimed at preventing the spread of plant parasitic nematodes.

Inter-cycle Management

The term 'inter-cycle' is used here to describe the time interval between the harvest of one crop and the planting of the next crop, which can vary greatly and thereby influence nematode management inputs. This is especially true when multiple crops are planted sequentially within a 12-month period (see Fig. 23.1).

Table 23.1. Nematodes considered to be important and/or exotic that could at some point be important to agriculture and therefore quarantine relevant. (From Handoo, 1998; Hockland *et al.*, 2006; EPPO, 2016).

Nematode	Host	Location
<i>Afrina wevelli</i>	<i>Eragrostis curvula</i>	South Africa
<i>Ditylenchus angustus</i>	Seed of rice	South-east Asia
<i>Ditylenchus gigas</i>	Legumes	North Africa
<i>Globodera ellingtonae</i>	Potato	USA
<i>Globodera zelandica</i>	Legumes	New Zealand
<i>Heterodera africana</i>	<i>Panicum maximum</i>	Ivory Coast
<i>Heterodera filipjevi</i>	Wheat, oats, barley	Various
<i>Heterodera graminis</i>	<i>Cynodon dactylon</i>	Australia
<i>Heterodera medicaginis</i>	<i>Medicago sylvestris</i>	Soviet Union
<i>Heterodera mediterranea</i>	<i>Pistacia lentiscus</i>	Italy
<i>Heterodera moths</i>	<i>Cyperus rotundus</i>	India
<i>Heterodera oryzae</i>	Rice	Ivory Coast
<i>Heterodera raskii</i>	<i>Cyperus bulbosus</i>	India
<i>Heterodera sorghi</i>	Sorghum	India
<i>Heterodera spinicauda</i>	<i>Phragmites australis</i>	Netherlands
<i>Meloidogyne coffeicola</i>	<i>Coffea arabica</i>	Brazil
<i>Meloidogyne ethiopica</i>	Vegetables	Africa, Brazil, Chile
<i>Meloidogyne enterolobii</i>	Vegetables, fruit	Various
<i>Meloidogyne fallax</i>	Potato, vegetables	Various
<i>Meloidogyne suginamiensis</i>	<i>Morus alba</i>	Japan
<i>Meloidogyne arabicida</i>	<i>Coffea arabica</i>	Costa Rica
<i>Meloidogyne lini</i>	Rice	China
<i>Meloidogyne kongi</i>	Citrus	China
<i>Meloidogyne hispanica</i>	<i>Prunus persica silvestris</i>	Spain
<i>Meloidogyne brevicauda</i>	<i>Camellia sinensis</i>	Sri Lanka
<i>Paratrichodorus</i> spp.	Potato, diverse crops	Various
<i>Pratylenchus loosi</i>	Tea, yam	Various
<i>Pratylenchus scribneri</i>	Diverse	Various
<i>Pratylenchus zeae</i>	Diverse	Various
<i>Thecavermiculatus</i>	<i>Oxalis tuberosa andinus</i>	Peru

Table 23.2. Nematodes considered economically important and present on quarantine lists worldwide. (From Hockland *et al.*, 2006.)

Nematode	Major host plants
<i>Anguina tritici</i>	Seed of wheat, barley
<i>Aphelenchoides fragariae</i>	Strawberry, ornamentals
<i>Aphelenchoides besseyi</i>	Seed of rice
<i>Bursaphelenchus xylophilus</i>	Pine trees
<i>Bursaphelenchus cocophilus</i>	Oil palm, coconut
<i>Ditylenchus dipsaci</i>	Seed of legumes, vegetables, cereals
<i>Ditylenchus destructor</i>	Potato, ornamentals
<i>Globodera pallida</i>	Potato
<i>Globodera rostochiensis</i>	Potato
<i>Heterodera glycines</i>	Soybean
<i>Heterodera schachtii</i>	Sugarbeet
<i>Meloidogyne chitwoodi</i>	Potato, vegetables
<i>Nacobbus aberrans</i>	Potato, tomato
<i>Radopholus similis</i>	Banana, ginger, citrus, pepper
<i>Xiphinema index</i>	Soil, grape
<i>Xiphinema americanum</i>	Soil, grape

Furthermore, in many instances, perennial crops (citrus, banana, sugarcane, coffee, papaya, etc.) are intercropped with annual crops that are hosts to one or more common nematode pests. This form of multilayered cropping is common in tropical regions of the world. In many instances, the number of crops in one field can increase significantly, greatly complicating targeted nematode management (Noe and Sikora, 1990). In tropical and subtropical vegetable production, for instance, as many as 11 short-cycle crops can be grown in a 12-month period. In some regions of South-east Asia, irrigated rice can be grown 3×/year, and favours root knot and lesion nematode population build-up. In India, large areas are planted to irrigated rice and wheat sequentially over a 12-month period without a break crop, and this can promote *Meloidogyne graminicola* development. Therefore, nematode management in the short inter-cycle periods between multiple susceptible crops can be extremely difficult, if not impossible.

In large commercial vegetable farms, effective soil fumigation can protect multiple crops grown sequentially in the same field from severe damage due to root knot nematodes. However, most small-scale and subsistence farmers do not

have the same access to expensive inputs, while in many cases resistant cultivars are not available. In addition, the rotation patterns that include sequential, relay, intercropping and, very often in subsistence agriculture, unsystematic randomized mixed cropping complicate developing management strategies. For example, the composition of cropping systems in many parts of the Sahel region in Africa is dominated by cropping patterns determined not only by environmental conditions but also by sociological and economic factors (Ker, 1995; Pearson *et al.*, 1995). In these situations, what is planted is selected to avoid risk of drought and pests and thereby ensure food for survival.

Intercropping annual crops in fields with perennial crops (e.g. coffee, banana, fruit trees) is also common. Often, both crops are good hosts of a common pest nematode and this affects both crops negatively, while making management complex and, in some cases, impossible (Fig. 23.3).

Management tools that can be used in the period between two susceptible crops, or when the damage threshold level is considered high, include resistant cultivars, nematicides and physical means of control, as well as crop-based cultural



Fig. 23.3. Intercropping banana with taro, both good hosts of root knot and lesion nematodes, in Tanzania. (Photograph courtesy of R.A. Sikora.)

practices, such as fallow, organic amendments, or the use of non-host break crops. However, management can also include combinations of approaches that often require information on nematode threshold densities, species composition, crop host spectrum and an economic analysis of the cost to the grower of using multiple inputs. In order to assess the distribution and importance of plant parasitic nematodes on crop yield in the tropics, unique experimental methods have been outlined by Noe and Sikora (1990). The complex interactions between environment, nematode and cropping patterns requires more research into the influence of cropping patterns on threshold densities, and thereby yield.

Physical management tools

Flooding

Nematode densities can drop significantly when soils are flooded for prolonged periods. In areas where paddy rice is flooded for prolonged periods, nematodes are often not a problem in the following dry-season crops (Bridge, 1996). Constant flooding of rice fields for 3 months or more gives acceptable control of root knot nematode for succeeding crops. The degree of root knot damage to processing tomato crops in the Philippines was undetectable in rotations of paddy rice–tomato (R.A. Sikora, unpublished data). Flooding, alternated with drying on a 2- to 3-week cycle during the summer, has been recommended for vegetables to reduce root knot nematode densities (Noling, 2003), and seems to be more effective than long, continuous flooding cycles. The duration of flooding for effective control may vary with the target nematode species. *M. graminicola* juveniles are killed after exposure to anaerobic conditions that begin in the soil a few days after flooding (Padgham *et al.*, 2003). The nematode will survive in waterlogged soil, however, for 14 months. *R. similis* can survive in bare soil in the absence of roots for 6 months, and can be controlled efficiently by flooding or planting banana after paddy rice. The duration of flooding for effective control needs to be determined for each nematode species. Significant levels of nematode control can also be attained when flooding is combined with other methods in an integrated management programme; for example,

with antagonistic cover crops (Fig. 23.4). In Taiwan, yearly sequential cropping of multiple short-season vegetables in between two short-term paddy rice plantings seems to be effective in keeping root knot nematode under control (R.A. Sikora, unpublished data). Depending on the crop production region, especially in drier climates with irrigated crops, the availability and cost of water for flooding-based nematode control needs to be considered.

Soil tillage

With the introduction of genetically engineered plants resistant to specific broad-spectrum herbicides, tillage has become almost non-existent in major crops like maize, soybean and cotton in some countries. The impact of non-tillage methodologies on free-living nematodes and plant parasitic nematode populations has been studied and is discussed in Chapter 3 of this volume. The elimination of alternate hosts and volunteer crop plants in the intercrop period due to the application of herbicides would have a positive effect on nematode reduction patterns. The higher level of organic matter would be expected to improve natural suppressiveness by stimulating biologically active antagonists, as discussed in Chapter 3, this volume, and by Sikora (1992).

In brief, tillage and residue management did not affect populations of *Rotylenchulus reniformis* in cotton (Molin and Stetina, 2013), but the integration of organic amendments and minimum tillage led to a reduction of root knot nematodes in vegetables (Stirling, 2013). Both zero tillage and residue retention in maize–wheat rotations in Mexico led to lower densities of *Pratylenchus thornei* and higher yields compared to conventional tillage practices. Zero tillage with rotation and residue retention enhanced water availability, soil structure and nutrient availability in wheat more than conventional tillage. Microbial diversity increased under zero tillage and residue retention, which may be useful for the biological control and IPM of nematodes (Govaerts *et al.*, 2006; Timper, 2014).

Where the practice is economical, repeated tilling of the soil at regular intervals for 30 days during hot and dry seasons between crops can reduce root knot nematode densities significantly in the upper horizons, due to desiccation of eggs and juveniles. Mathur *et al.* (1987)



Fig. 23.4. Root knot nematode management using irrigated rice rotated with sunn hemp before vegetables and legume crops in Myanmar. (Photograph courtesy of R.A. Sikora.)

reported that one to five deep ploughings reduced *Heterodera avenae* populations by 9–42%. However, more than one ploughing is probably uneconomical. Tillage reduces densities of the target nematode pest as well as secondary pest species, and it will also eliminate alternative weed hosts and volunteer plants from the previous crop. However, one weed host or one volunteer host plant from the previous susceptible crop is often sufficient to maintain a nematode population at threshold densities. Soil tillage and careful mounding-up of the thin top layer of soil into ridges for tobacco beds gave good control of root knot. The upper 5 cm of soil heats to 36°C in the dry season and has only 1% moisture, which leads to total nematode desiccation (Ferris, 1969).

Clean fallow

Fallows in plant-free fields are seldom practised due to problems with soil erosion and the simple fact that it is more economical to produce a short-season crop. In addition, clean fallow requires either additional tillage to kill weeds or the use of herbicides, which are both cost factors. Such

fallows reduce nematodes more effectively than a field with weed cover, due to the exclusion of alternative host plants. Clean fallows are most effective in nematode management in the hot, dry summer months between crops. Clean fallow breaks between field and vegetable crops have been shown to reduce root knot nematode populations by up to 90% (Roberts *et al.*, 2005). In some production systems, setting aside land as clean fallow has been practised, where more land is available than that needed to plant, such as in the western USA. The negative effects on soil conservation will, however, limit the use of clean fallow in many countries.

Organic amendments

Organic amendment is used here to mean organic material incorporated into the soil that comes from external sources, such as processing residues or industrial waste products. Organic materials added as fresh crop residue and grown in the rotation – break, cover, trap, antagonistic or green manure crops – are discussed in another section below. Incorporation into the soil of large amounts of any organic material will reduce

nematode densities. Crop residues, oil cakes, coffee husks, paper waste, crustacean skeletons, sawdust and chicken manure, to name a few, have been used with some success (Figs 23.5, 23.6 and 23.7).

Control may be due to any one or more of the following mechanisms:

- Toxic compounds present in the organic material;

- Toxic metabolites produced during microbial degradation;
- Enhancement of soil antagonist activity by promoting microbial and invertebrate communities;
- Exposure to lethal temperature where plastic mulch is also used.

A list of some of the more common organic amendments used for nematode control is given



Fig. 23.5. Combining a non-host cover crop with solarization and nematode-free tissue culture planting material for *Radopholus similis* management in banana in Costa Rica. (Photograph courtesy of R.A. Sikora.)



Fig. 23.6. The use of sesame straw as a mulch with raised beds and with drip irrigation under plastic tunnels in vegetable production in the Sinai, Egypt. (Photograph courtesy of R.A. Sikora.)

in Table 23.3. Chitin amendments have received much interest in the past as an organic amendment in that they stimulate the antagonistic potential in soil toward nematodes (Culbreath *et al.*,

1985; Rodriguez-Kábana *et al.*, 1987; Spiegel *et al.*, 1987). Organic amendments have also been combined with various biocontrol agents, with reports of enhanced levels of control. The use of



Fig. 23.7. Mulching with coffee husk for root knot nematode management in short-cycle sequential organic vegetable production in the Philippines. (Photograph courtesy of R.A. Sikora.)

organic amendments is often limited by availability, and in some cases, by the large quantities needed to be effective. In addition to their effects on nematode population density, organic amendments also improve soil structure and water-holding capacity, reduce diseases and limit weed growth, all of which ultimately lead to a stronger plant and improved tolerance to nematode attack.

Crop-based management tools

Crop management tools are designed to attain high yield while simultaneously suppressing nematode, insect, disease and weed problems, reducing erosion and improving soil fertility. Each production system has different requirements when it comes to combatting nematode infestations. In addition, the rotation crops used

Table 23.3. Important organic soil amendments used for nematode control.

Oil cakes	Agro-industrial wastes	Animal and urban waste	Plant residues
Margosa/neem	Sawdust and tree bark	Chicken manure	Water hyacinth
Mustard	Cellulose waste	Farmyard manure	Seaweed
Groundnut	Sugarcane bagasse	Garden compost	Margosa/neem leaves
Sesame	Sugarcane filter cake	Fish remains	Cabbage leaves
Castor	Rice and coffee husks	Bonemeal	Pineapple leaves
Mahuva	Wood ash	Crustacean skeletons	
Cotton seed	Cotton waste	Raw sewage	
Soybean	Cassava peelings	Refuse	
Linseed	Cocoa pods	Urea	
	Tea waste		
	Mycelium waste		
	Potato processing water		
	Sugarbeet processing water		

by a grower are planted for different reasons, with the type of rotation crop varying greatly between the tropics and the subtropics. Selection is often dependent on the main cash crop and economics of other crops in the cropping system. Rotation crops are used to:

- Suppress weed growth;
- Prevent soil erosion;
- Improve soil organic matter levels;
- Increase water-holding capacity;
- Raise nitrogen concentration directly;
- Control nematodes and other soil-borne pathogens.

Nematode control achieved with crop management is attained by several mechanisms, including starvation, trapping, antagonism, stimulation of soil antagonistic potential and/or different degrees of biofumigation. Organic production systems are heavily reliant on the benefits of crop rotation. Conversely, in commercial production of many horticultural crops, where fumigation is the backbone of the cropping system and sequential cropping of susceptible vegetable crops is practised, rotation may not be relevant.

Weed fallow

In a normal fallow, weed growth is usually not managed and often leads to extensive biomass production. However, if a few weed species are good hosts for the pest nematode, or sufficient volunteer plants of the preceding susceptible

crop are present, nematode densities may actually increase during the fallow period. Mulching of the weeds prior to planting of the next crop stimulates the antagonistic potential in the soil and leads to a reduction in inoculum densities. Such fallows are common in the tropics during the rainy periods between major crops. Incorporation and solarization of these weeds has been shown to lead to a significant reduction in root knot in horticultural crops.

Non-host crops

Non-host crops are defined here as crops harvested for marketing purposes, as opposed to cover crops used for soil conservation, animal grazing or direct nematode control. Rotation with non-host crops is the most important technique used for root knot management worldwide and has been discussed in detail elsewhere (Nusbaum and Ferris, 1973; Barker, 1991; Rodriguez-Kábana, 1992; Noe, 1998). In [Table 23.4](#), a list of primary hosts, host range size and some acceptable rotation crops is given. It should be noted that where multiple nematode parasites are present, a non-host crop used for management of one species may be a good host for the non-target species. Since host susceptibility can also vary among the populations of a species, testing of a non-host is always warranted before making final recommendations. In the past, many crops considered to be non-hosts of a nematode were found to be moderate hosts.

Table 23.4. A partial list of primary hosts and non-host crops used in rotations for nematode management. (From Noe, 1998; Anon., 2004; CABI Crop Protection Compendium; other chapters in this volume.)

Nematode	Primary hosts	Rotation crops ^a
<i>Meloidogyne incognita</i>	Horticultural crops, cotton, soybean, legumes	Groundnut, T and R cvs., some cereals
<i>Meloidogyne graminicola</i>	Rice, wheat	Legumes, soybean, jute, sunflower, sweet potato, sesame, okra
<i>Meloidogyne chitwoodi</i>	Horticultural crops, potato, carrot	Lucerne
<i>Meloidogyne javanica</i>	Horticultural crops, especially tomato, legumes	Cotton, groundnut
<i>Meloidogyne arenaria</i> Race 1	Groundnut	Cotton, maize, sorghum
<i>Meloidogyne arenaria</i> Race 2	Horticultural crops, soybean	Cotton, groundnut, maize, sorghum
<i>Meloidogyne hapla</i>	Horticultural crops, especially carrot, celery, potato, lucerne	Onion, lettuce, radish
<i>Meloidogyne artiellia</i>	Chickpea, vegetables	Cotton, potato, oat, maize, lentil, tomato, melon
<i>Heterodera glycines</i>	Soybean	Cereals, most legumes, R cvs.
<i>Heterodera schachtii</i>	Sugarbeet, cabbage, brussels sprouts, cauliflower, broccoli, rape	Cereals, T and R cvs., horticultural crops
<i>Heterodera cajani</i>	Pigeonpea, chickpea	Cereals
<i>Heterodera avenae</i>	Cereals	Legumes, potato, sugarbeet, horticultural crops
<i>Heterodera ciceri</i>	Chickpea	Cereals, cotton, horticultural crops
<i>Heterodera oryzae</i>	Rice	Legumes
<i>Globodera rostochiensis</i>	Potato, tomato	Cereals, legumes, R cvs.
<i>Hirschmanniella</i> spp.	Rice	Legumes, cereals, cotton, tobacco, sweet potato
<i>Punctodera chalconensis</i>	Maize, teosinte	All other grasses and cereals
<i>Scutellonema bradys</i>	Yams	Groundnut, chilli pepper, tobacco, cotton, maize, sorghum
<i>Globodera pallida</i>	Potato, tomato	Cereals, legumes, R cvs.
<i>Ditylenchus dipsaci</i> oat race	Onion, garlic, legumes, oats	Some cereals, horticultural crops
<i>Ditylenchus gigas</i>	Broadbean	Cereals, horticultural crops

Note: ^aT = tolerant; R cvs. = resistant cultivars.

Rotation with non-hosts can affect the rate of natural attrition and, therefore, the extent of nematode inoculum reduction between susceptible crops. In sugarbeet production, barley reduces *Heterodera schachtii* to a greater degree than wheat when used as a non-host crop in rotation. Nematode survivability over time in the absence of a host is also very important in designing rotation schemes. Some nematodes can survive long periods in the absence of a host (e.g. *Xiphinema*, *Heterodera*, *Globodera*), whereas other nematodes (e.g. *Rotylenchulus*, *Meloidogyne*, *Nacobbus*) decrease more rapidly over time. A list of the duration of survival under different conditions has been made by Norton (1978) and is discussed in the various chapters in this book. Survival periods

for some selected nematode species are given in Table 23.5.

Rotations using moderately resistant or tolerant crops together with highly susceptible vegetable crops have been used for the control of root knot. Vegetables considered moderately susceptible or tolerant to root knot include cabbage, onion, leek, broccoli and amaranth. Plants considered good host plants of one *Meloidogyne* species in one part of the world are not necessarily hosts to all populations of that species. Because of this large variation in host status within species of root knot, all crops being considered for rotation must be tested for host status to local populations before rotation schemes are recommended. Caution must be taken with regard to variation in nematode populations and to the

Table 23.5. Duration of survival of some plant parasitic nematodes in the absence of a host plant.

Nematode	Survival without a host plant
<i>Anguina tritici</i>	28 years in seed at room temperature
<i>Aphelenchoides besseyi</i>	1–3 years in dry rice seed
<i>Criconemoides xenoplax</i>	2 years in flooded soil
<i>Ditylenchus angustus</i>	4 months in flooded soil
<i>Ditylenchus dipsaci</i> , <i>D. gigas</i>	Many years in dry seed
<i>Globodera rostochiensis</i> , <i>Globodera pallida</i>	10–15 years in infested soil
<i>Heterodera glycines</i>	7–8 years in infested soil
<i>Meloidogyne</i> spp.	1–12 months
<i>Meloidogyne graminicola</i>	5 months in flooded soil
<i>Pratylenchus coffeae</i>	6 months in bare soil
<i>Radopholus similis</i>	6 months in bare soil
<i>Rotylenchulus reniformis</i>	2 years in bare soil; 1.5 in dry soil
<i>Xiphinema americanum</i>	1 year in soil at 10°C

composition of root knot species present in a field. Sometimes, the *Meloidogyne* populations are composed of several species that may require different approaches for control. It should also be noted that detection of species that make up less than 5% of the population is difficult.

Multiple cropping and mixed cultivars

Multiple cropping is common in subsistence agriculture where food for family consumption is the primary goal. The simultaneous production of many different crops increases the chances of obtaining a crop regardless of environmental calamities such as serious drought or pest and disease occurrence. The multiple-cropping systems used do not necessarily lead to a reduction in nematode damage, since spacing between susceptible crops is often small (Noe and Sikora, 1990). In some cases, good host perennial crops are grown in those fields that maintain nematode populations such as root knot (see Figs 23.2 and 23.3).

The use of alley cropping, on the other hand, could reduce nematode damage in multiple cycles of crops in a year if the survival of the nematode in question (see Table 23.5) is limited and the length of the growing season in the two or more crops grown in distinct alleys is sufficiently long. Alley cropping with a cereal in one alley and a susceptible crop in the alternating alley and rotation of the crops in these sections after the first cycle could lead to sufficient reductions of nematode densities. Alley cropping of resistant and susceptible vegetable cultivars could also be an approach to both reduce nema-

tode densities and offset the development of resistance-breaking populations. Alley cropping with high-value crops using bare fallow, trap crops or antagonistic fodder crops used for grazing in the alley also needs to be examined. In banana, alley cropping with an alternating fallow seems to have been successful. The use of precision agricultural technology and remote sensing should allow the growers of some crops to plant resistant cultivars in field areas with high nematode infestations; for example, in crops such as cotton, soybean, wheat, sugarbeet and potato.

Trap crops

Trap cropping normally targets sedentary nematodes. A good host with quick and extensive root growth is planted for a short duration. The crop and planting period must be selected to ensure high nematode penetration and initial development to a non-motile growth stage, usually only a few days after root penetration. The sedentary juveniles in the root tissue are then killed when the trap crop is terminated by physical destruction or herbicide treatment. Trap cropping, which was originally developed to control cyst nematodes in sugarbeet in the 1800s, has now been used for nematode management in a number of crops (Table 23.6). Short-season crops are used as trap crops in raised beds in Cuba to control *Meloidogyne* species, and rape has been used as a green manure crop to reduce *H. schachtii*. Resistant mustard and oil radish cultivars are also used as a trap crop for the management of sugarbeet cyst and root knot nematodes in beet crops.

Table 23.6. Nematode trap crop approaches used in the field for nematode management.

Nematode	Trap crop	In rotation
<i>Heterodera schachtii</i>	<i>Sinapis alba</i> , <i>Raphanus sativus</i> ssp. <i>oleifera</i>	Sugarbeet, brassica crops and cereals
<i>Meloidogyne incognita</i>	<i>Solanum nigrum</i> <i>Lactuca sativa</i> , <i>Raphanus sativus</i>	Monoculture of African spinach Break crop in protected cultivation
<i>Globodera rostochiensis</i>	<i>Solanum tuberosum</i> , <i>Solanum</i> <i>sisymbriifolium</i>	Potato
<i>Globodera pallida</i>	<i>Solanum sisymbriifolium</i>	Break crop in potato rotation
<i>Heterodera avenae</i>	<i>Avena sativa</i>	Wheat, barley

Trap crops stimulate hatch and penetration, and reduce nematode density both by trapping and, where present, by resistance mechanisms. They also stimulate the antagonistic potential after biomass incorporation into the soil. Any host that can be planted and then killed by incorporation or herbicide application can be used as a trap crop. However, the following criteria are important for successful trap cropping:

- Excellent host with extensive root growth to ensure high levels of penetration;
- Low-cost seed, since yield and/or a green manure effect are not always expected;
- Good data on 'day degrees' from penetration until start of nematode egg laying;
- Speedy and complete kill of the root to prevent any reproduction after incorporation;
- Acceptable cost–benefit ratio.

It should be noted that if root removal or herbicide killing of the plant is undertaken too close to the start of egg laying, the females in the surviving root or dying root tissue can lay eggs for a number of days, thereby reducing control efficacy. Therefore, adequate soil tillage to promote root death or the use of herbicides that systemically kill root tissue are needed for effective management.

Cover crops

Cover crops are non-hosts that are used mainly to protect the soil from erosion or to suppress weed growth between major crop cycles, or crops used to give some nematode control. They may also be used for animal fodder or as a green manure crop. Cover crops reduce nematode densities by being non-hosts. However,

when incorporated into the soil, they can increase the antagonistic potential in the soil significantly (Sikora, 1992). In addition, microbial degradation of organic compounds leads to the production of metabolites that are nematicidal as a form of biofumigation. Major cover crops that have been tested for use are given in Table 23.7.

Antagonistic crops

Plants antagonistic to nematodes are those that are considered to produce antihelminthic compounds with different modes of action (Pandey *et al.*, 2003). The mechanisms responsible for control are often poorly understood, and many of the tests made have been conducted *in vitro* with plant extracts. The production and active release of toxic substances while the crop is growing or after incorporation into the soil is usually responsible for control. A large number of plants have been shown to contain nematicidal compounds when extracted from the tissue and tested *in vitro*.

Marigold (*Tagetes* spp.), sunn hemp (*Crotalaria juncea*), castorbean, partridge pea, asparagus and sesame have been studied extensively for nematode control activity. Sunn hemp is often used as a cover crop and green manure crop, and is sometimes considered an antagonistic crop for root knot nematode control. *Crotalaria longirostrata*, for example, when grown as a cover crop and then incorporated into the soil, will reduce root knot galling. Control is probably due to toxins produced during microbial degradation and not by toxic exudates from the plant itself. In Fig. 23.8, *Crotalaria juncea* is shown, used to control root knot nematodes in many crops. The best-studied antagonistic

Table 23.7. Major cover crops used for nematode management.

Nematode	Cover crop
<i>Belonolaimus longicaudatus</i>	<i>Crotalaria spectabilis</i> <i>Tagetes minuta</i>
<i>Heterodera schachtii</i>	<i>Fagopyrum esculentum</i> <i>Phacelia tanacetifolia</i>
<i>Hirschmanniella oryzae</i>	<i>Sesbania rostrata</i> <i>Sphenoclea zeylanica</i>
<i>Meloidogyne</i> spp.	<i>Aeschynomene americana</i> <i>Chloris gayana</i> <i>Crotalaria juncea</i> <i>Crotalaria spectabilis</i> <i>Crotalaria intermedia</i> <i>Desmodium uncatatum</i> <i>Digitaria decumbens</i> <i>Eragrostis curvula</i> <i>Festuca pratensis</i> <i>Mucuna pruriens</i> <i>Mucuna deeringiana</i> <i>Panicum maximum</i> <i>Sorghum bicolor</i> × <i>S. bicolor</i> var. <i>sudanense</i> <i>Stylosanthes gracilis</i>
<i>Meloidogyne arenaria</i>	<i>Paspalum notatum</i>
<i>Meloidogyne chitwoodi</i>	<i>Raphanus sativus</i>
<i>Meloidogyne incognita</i>	<i>Brachiaria plantaginea</i> <i>Cynodon dactylon</i> <i>Macroptilium atropurpureum</i> <i>Panicum maximum</i> <i>Pennisetum purpureum</i> <i>Raphanus sativus</i> ssp. <i>oleifera</i>
<i>Pratylenchus brachyurus</i>	<i>Crotalaria usaramoensis</i> <i>Stylosanthes gracilis</i> <i>Flemingia congesta</i>
<i>Pratylenchus loosi</i>	<i>Tripsacum laxum</i> <i>Cymbopogon confertiflorus</i> <i>Eragrostis curvula</i>
<i>Pratylenchus neglectus</i>	<i>Raphanus sativus</i>
<i>Rotylenchulus reniformis</i>	<i>Chloris gayana</i> <i>Crotalaria juncea</i> <i>Tagetes patula</i>

plants are species in the genus *Tagetes* (marigold), known to produce alpha-terthienyl and derivatives of bithienyl that are toxic to root knot. Ploeg (2002) demonstrated that *Tagetes patula*, *Tagetes erecta*, *Tagetes signata* and a *Tagetes*

hybrid reduced galling in a subsequent susceptible tomato crop compared to a tomato–tomato rotation. *Tagetes* has been used to suppress nematodes by various mechanisms, including acting as a poor host, enhancing nematode-antagonistic microorganisms, or acting as a trap crop. A comprehensive review of the use of *Tagetes* plantings for nematode control is provided by Hooks *et al.* (2010).

Biofumigation

This term normally refers to the suppression of soil-borne pests and pathogens by biocidal compounds, principally isothiocyanates, released in soil when glucosinolates in cruciferous crop residues are hydrolysed (Kirkegaard *et al.*, 1998). Soil amended with fresh or dried cruciferous residues at 38°C day and 27°C night temperatures reduced *Meloidogyne incognita* galling by 95–100% after 7 days' incubation in controlled environment tests (Stapleton *et al.*, 1998). It should be noted here, however, that many cruciferous plants are good hosts of some important species of *Meloidogyne* and cyst nematodes, such as *H. schachtii* and *Heterodera cruciferae*.

The term 'biofumigation' is now used more freely whenever volatile substances are produced through microbial degradation of animal and plant organic amendments that result in significant toxic activity toward a nematode or disease (Bello, 1998; Ploeg, 2008). The release of toxic compounds already present in antagonistic plants used as amendments, e.g. neem, marigold and castor, or the production of toxic compounds due to microbial fermentation of nutrient-rich organic amendments, e.g. velvet bean, sunn hemp or elephant grass, lead to significant levels of nematode control.

Biofumigation under these circumstances is greatest when there is an optimum combination of organic matter, high soil temperature and adequate moisture to promote microbial activity leading to toxin production. Ploeg and Stapleton (2001) reported that biofumigation with broccoli failed to control *M. incognita* or *Meloidogyne javanica* at 20°C, but resulted in near-complete control at temperatures of 30–35°C. In tropical and subtropical production systems, plastic mulch and drip irrigation improve the effectiveness of biofumigation.



Fig. 23.8. *Crotalaria juncea* cover crop and green manure, often used for its antagonistic effects on root knot nematodes. (Photograph courtesy of E. Stein, Tropical Seeds, USA.)

Control due to any form of biofumigation is probably the result of multifaceted mechanisms, including:

- Non-host or trap cropping depending on the host status of the plant used;
- Lethal temperature due to fermentation;
- Nematicidal toxic by-products produced during organic matter degradation;
- Stimulation of antagonists in the soil after biofumigation.

Pre-plant Management

Management tools used just prior to sowing or transplanting can have a major impact on plant health in the early stages of plant growth. These methods are designed to offer protection from infection for 4–5 weeks after germination or transplanting in the critical root pathozone (Fig. 23.9).

Disruption of nematode activity in the pathozone by suppressing specific infective stages

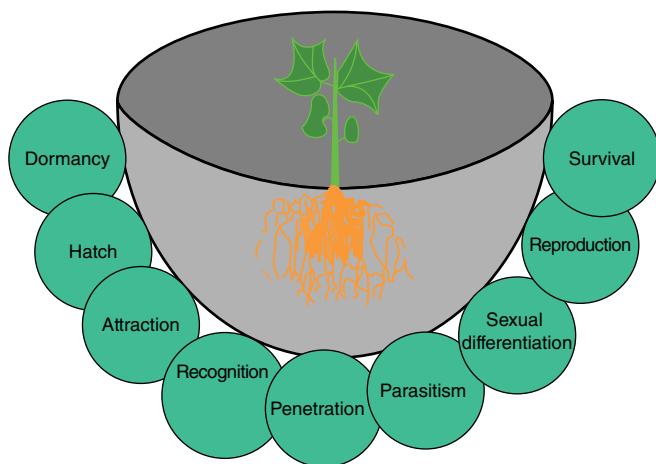


Fig. 23.9. The highly sensitive early developing root system of a host plant and the behavioural phases in the life cycle of a nematode that are targeted for management in the pathozone.

of the nematode life cycle is an effective means of management (Sikora *et al.*, 2007). In most cases, the nematode does not have to be killed, but penetration into roots must be delayed. Any treatment that prevents the nematode from entering the developing root system has important plant protection potential. Depending on the management tool used, completely different modes of action are involved in suppressing nematode infection, as shown in Fig. 23.9.

Precision and remote sensing

Significant progress has been made in the use of remote sensing technology for temporal and spatial mapping of nematodes, foliar diseases and weeds in field crops that can be used for subsequent pest management (Oerke *et al.*, 2010). A range of modern sensor technology, based on infrared thermography and hyperspectral reflectance, has been developed that allows the detection of nematode damage from helicopters, airplanes and drones (Nicolas *et al.*, 1991; Nutter *et al.*, 2002; Schmitz *et al.*, 2004; Hillnhütter *et al.*, 2010, 2011).

Nematode distribution in a field is usually random and clustered, with damage intensity usually seen as foliar malfunctioning, correlated with initial population density (Pi) measured just before planting. An example is shown in Fig. 23.10 using infrared aerial hyperspectral imaging and image classification. Hyperspectral sensors can be used to detect clustered distribution

by measuring nematode-induced changes in crop canopy chlorophyll reflection, which can be correlated with soil sampling to determine population densities in the damage areas (Fig. 23.11). The clustered occurrence and low level of nematode mobility in soil and their induction of symptoms in the leaves make them perfect targets for remote sensing technology and follow-up management. The data obtained can be used for site-specific nematicide application, or for the planning of rotations with non-hosts or trap crops (Hillnhütter *et al.*, 2010, 2011).

Research is needed that links remote sensing information with data on nematode population dynamics over different rotational patterns and with biological and environmental factors to develop computer models for decision making at the farm level (Schmidt *et al.*, 1993; Fricke *et al.*, 2014). Once this link is established, IPM computerized extension programs can be provided to the growers for nematode management, such as Nemaplot (Schmidt, 2017).

Soil electrical conductivity (SEC) technology used to determine soil textures, coupled with GPS-GIS technology, has been developed that now allows the accurate, efficient and economical development of georeferenced soil texture maps that facilitate site-specific nematicide application systems (Mueller *et al.*, 2010), see Figs 23.12 and 23.13. Remote sensing technology coupled with precision farming equipment can increase control efficacy; for example, by allowing the



Fig. 23.10. AISA hyperspectral false colour infrared picture at GS 31 and digital map of *Heterodera schachtii* damage clustering. (a) Infrared photograph; (b) digitalized computer map with colours correlated with final nematode (Pf) densities. Class 1 (healthy = red); Class 2 (Pf < 1000 eggs and J2/100 ml soil = green); Class 3 (Pf > 1000 eggs and J2/100 ml soil = blue); Class 4 (RCRR-rating < 3 = yellow); Class 5 (RCRR-rating > 3 = cyan). RCRR = Rhizoctonia crown and root rot. (From Hillnhütter *et al.*, 2011.)

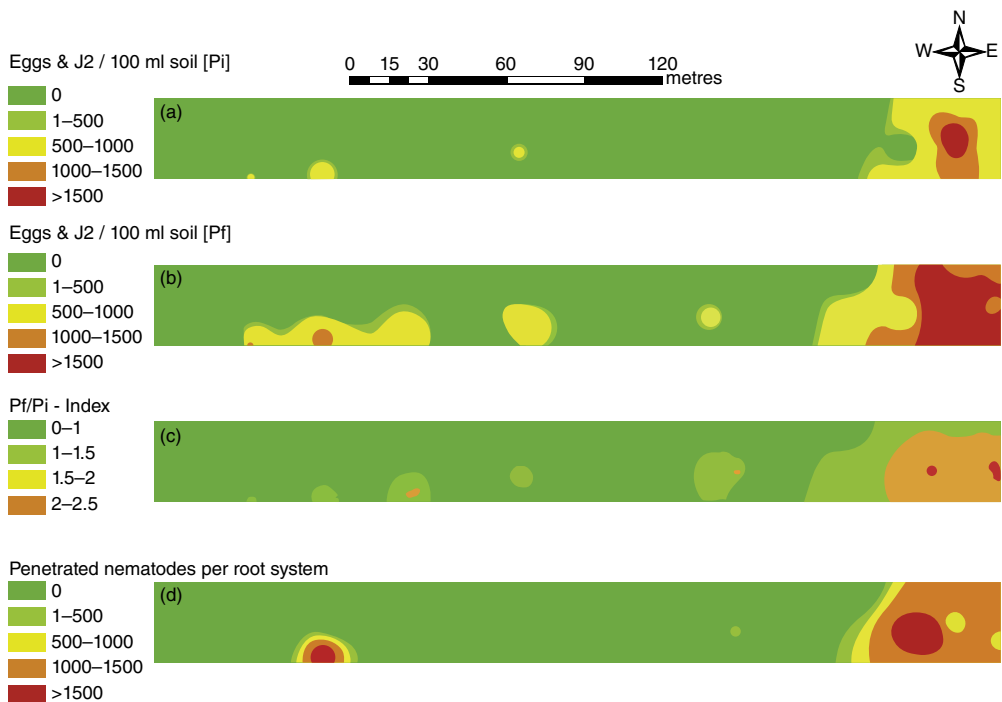


Fig. 23.11. Spectral Angle Mapper classification of *Heterodera schachtii*: Pi and Pf, Pf/Pi Index and nematode penetration/root system generated from the AISA hyperspectral false colour infrared picture of the infested field shown in Fig. 23.10.)

placement of nematicides in field areas of high nematode density. Using the same technology, selective seed or transplant placement of susceptible or resistant cultivars in field areas with low or high nematode densities would reduce costs for resistant cultivars and lessen the overall rate of selecting resistance-breaking nematode populations. Mapping fields in this manner could

also be used for optimizing rotation plantings for nematode management.

Solarization and biofumigation

The lethal temperature for the control of plant parasitic nematodes is considered to be around



Fig. 23.12. Soil electrical conductivity – EC_a meter (GPS) attached to the back of a truck for measuring soil texture distribution in a cotton field in North Carolina, USA. (Photograph courtesy of C. Holguin, Clemson University.)

45°C. Heating the soil either with dry or steam heat has been used for many years in protected cultivation to manage root knot nematodes, but the high cost of heating oil has limited its use drastically. Soil solarization with clear mulches (Fig. 23.14), which leads to the development of lethal temperatures in the soil, has been recommended for reducing densities of

root knot and soil-borne disease organisms (Katan, 1981; Whitehead, 1998). The technique is most effective in regions where high levels of solar energy are available for extended periods. However, the limited depth to which lethal heat penetrates soil often restricts control to the uppermost 5–10 cm. Therefore, this is not a stand-alone management tool

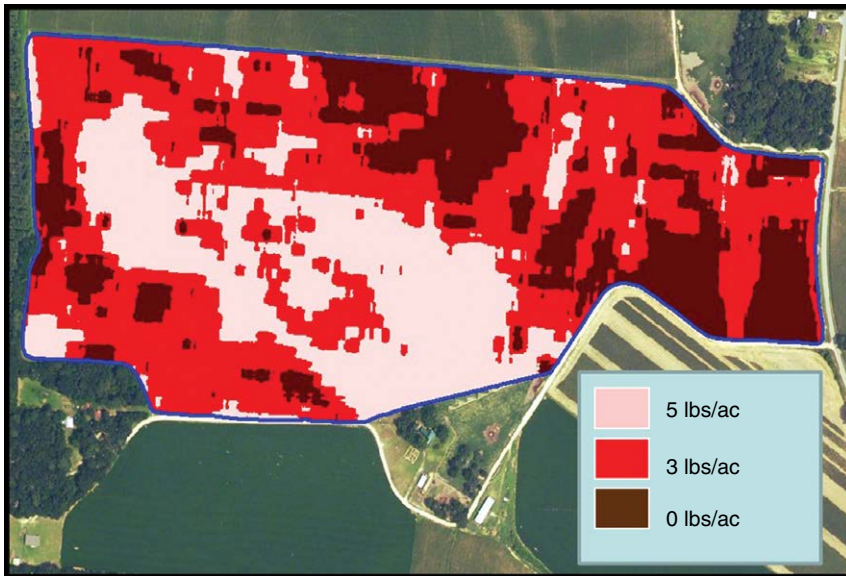


Fig. 23.13. Prescription map showing differences in soil texture, generated from soil electrical conductivity equipment and used for site-specific and dose-specific nematicide application to a cotton field infested with *Rotylenchulus reniformis*. (Photograph courtesy of C. Holguin, Clemson University.)



Fig. 23.14. Solarization of vegetable beds with plastic mulch for root knot nematodes and *Fusarium* wilt management under high solar radiation in the Jordan Valley. (Photograph courtesy of H. Saleh.)

and needs to be combined with other control measures.

Solarization will reduce root knot, *Verticillium* and *Fusarium* wilt and weeds in crops, even though climatic conditions are not considered ideal for soil solarization (Overman and Jones, 1986). Similar results were obtained in Cuba in peri-urban agriculture and in small farm production units using solarization under suboptimal conditions between July and September (Fernández and Labrada, 1995). The high cost of plastic mulches is a limiting factor in subsistence agriculture, except when used to control nematodes in soil for seedbeds or pot plantings (Bridge, 1987, 1996). The integration of solarization with organic mulches to improve the effectiveness of biofumigation has been used in the management of root knot in vegetable production. The combination improves biofumigation and the production of toxic compounds in the soil. For example, the combination of solarization for 30 and 60 days with incorporation into the soil of 5000 kg/ha of *Calotropis procera* resulted in significant reduction in *M. incognita* root galling and a simultaneous yield increase of melon (Lugo *et al.*, 2010).

The combination of fresh marigold as an amendment and solarization is used effectively for root knot management in protected cultivation in Morocco. *Tagetes* is grown in raised beds prior to the planting of susceptible horticultural crops. The fast-growing *Tagetes* is incorporated into the soil after 2–3 months. The beds are fitted with drip irrigation and covered with a plastic mulch. The soil in the bed is then biofumigated under conditions of high summer soil temperature and optimum soil moisture for heat penetration (Fig. 23.15).

Nematicides

Fumigant nematicides are used to control plant parasitic nematodes before planting. In most cases, fumigants are broad-spectrum contact nematicides effective against adults, juveniles and eggs, as well as other pests and diseases, plus weeds. Several excellent reviews on the use of the most common fumigant and non-fumigant nematicides for a broad array of nematodes and crops, are recommended for more detail (Johnson, 1985; Whitehead, 1998; Noling, 2003;

Anon., 2004; Haydock *et al.*, 2013; Becker, 2014) and in the chapters in this volume. The most commonly used fumigant nematicides are listed in Table 23.8. In many systems, fumigants are applied under plastic mulch and transplants inserted later through the plastic into raised beds (Fig. 23.16).

Due to the often simultaneous occurrence and negative impact of nematodes, weeds and soil fungi on production in many growing areas, a broad-spectrum fumigant is highly desirable, especially where multiple susceptible crops are grown sequentially. When used as directed, fumigants will give excellent nematode control and increase yield significantly. Because registration requirements and efficacy vary with country and crop, no attempt will be made here to list those still being used for the control of nematodes in particular root knot nematodes in vegetables. The majority of smallholder farmers, especially subsistence farmers, usually cannot use fumigants because of a lack of capital for equipment and the nematicide. In developed commercial agriculture, many limitations have been placed on fumigation near urban dwellings, including significant fumigation-free buffer zones.

Non-fumigant nematicides are granular or liquid formulations that enter a water-soluble stage in the soil and have either contact or nematostatic action. They also often have additional plant-systemic activity against nematodes, and in some cases insects and even fungal pathogens. The non-fumigant nematicides on the market are listed in Table 23.8. As product registration requirements vary between countries, it is important that these are followed closely. The list is to be used as a reference and not as a recommendation by the authors. The use of non-fumigant nematicides is also discussed, where applicable, in respective crop chapters in this volume.

In most cases, the mechanism of action is associated with the suppression of nematode mobility during the period when adequate concentrations are in the soil solution. Some of the newer nematicides have activity toward the mitochondria of the nematode and cause direct mortality. The non-fumigant nematicides are not effective against the eggs of nematodes, and in some cases do not kill the juveniles at the concentrations now being recommended for use. They give the plant a 'head start' by delaying



Fig. 23.15. From left to right: elevated beds planted to marigold (*Tagetes* sp.) as an antagonistic plant, incorporation as a green manure followed by biofumigation under plastic mulch with additional drip irrigation for the control of *Meloidogyne incognita* in large-scale vegetable production greenhouses in Morocco. (Photograph courtesy of H. Kaak and R.A. Sikora.)

nematode penetration during the highly sensitive seedling or post-transplant stage of plant development (Fig. 23.9). Non-fumigant granular and/or liquid formulations of contact and/or systemic nematicides are suitable for commercial use as well as for use on small farms. Growers, however, must be trained in proper handling and application techniques, as well as the time of application, since many of these materials are highly toxic to humans and the environment. Non-fumigant nematicides are often not as effective as fumigants in increasing yields, because they do not have broad-spectrum activity and in some cases only inactivate nematodes for short periods.

Granular nematicides are either applied broadcast over the soil surface and incorporated into the soil before planting or banded into or over the plant furrow. The presence of residues in the harvested crop is possible if treatment instructions are not followed.

Liquid formulations allow application by surface and drip irrigation, with the latter of importance to vegetable, tree and vine production. Application through drip irrigation places the material directly in the rhizosphere and can allow treatment during the growing season. It also allows splitting or extending application over specific time intervals to coincide with optimum control. However, many non-fumigants, while effective in preventing infections, are not highly effective in suppressing nematode activity

once infection has occurred. Dip treatment or the treatment of vegetable transplants in nurseries has also been effective in reducing root knot galling.

Seed treatment is now widely used, and advances in the development of new formulations that allow seed treatment for nematode management are available for many crops. Seed treatment significantly reduces the dose needed per unit area, reducing environmental impact and residue problems. In many agronomic and vegetable crops, protection of the roots for 4–5 weeks can provide significant nematode control and boost yield (Becker, 2014). Seed treatment with combinations of nematicides with other pesticides is now a standard practice on many field crops, permitting targeted IPM of nematodes and diseases in the pathozone in the early stages of plant growth.

In recent years, many nematicides have been removed from the market or phased out due to unacceptable non-target toxicity (Haydock *et al.*, 2013). Despite the adverse impact, nematicides remain one of the most effective methods of controlling nematodes, often as part of ICM strategies (Sikora *et al.*, 2005). In Australia, no nematicides are currently registered for the control of sugarcane nematodes after the phasing out of organophosphorus nematicides. In South Africa, carbofuran, oxamyl and furfural aldehyde are registered for use on sugarcane. New

Table 23.8. Fumigant, non-fumigant and biological commercial products used for the management of plant parasitic nematodes.^a (From Becker, 2014.)

Common name	Active ingredient(s)	Chemical class/organisms
Terr-O-Gas, Tri-Con, Brom-O-Gas	Methyl bromide	Halogenated hydrocarbon
Cloropicrin 100 Fumigant, Metapicrin	Chloropicrin	Halogenated hydrocarbon
Telone II, Telone EC	1,3-dichloropropene (1,3-D)	Halogenated hydrocarbon
Telone C-17, C-35, InLine	1,3-D plus chloropicrin	Halogenated hydrocarbon
Midas	Methyl iodide	Halogenated hydrocarbon
Dominus	Allyl isothiocyanate	Mustard oil
Enzone	Sodium tetrathiocarbonate	Thiocarbonate
Paladin	Dimethyl disulfide	Organosulfur
Temik, Meymik	Aldicarb	Carbamate
Vydate	Oxamyl	Carbamate
Aeria ^c	Thiodicarb	Carbamate
Metam, Vapam, Sectagon 42	Metam sodium ^b	Dithiocarbamate
K-Pam HL, Sectagon K54	Potassium sodium ^b	Dithiocarbamate
Basamid	Dazomet ^b	Dithiocarbamate
Counter	Terbufos	Organophosphate
Nemacur	Fenamiphos	Organophosphate
Nemathorin, Eclahra	Fosthiazate	Organophosphate
Nemakick	Imicyafos	Organophosphate
Mocap	Ethoprop	Organophosphate
Rugby, Apache	Cadusaphos	Organophosphate
Admire Pro	Imidacloprid	Neonicotinoid
Movento	Spirotetramat	Tetramic acid
Nimitz	Fluensulfone	Fluoroalkenyl (-thioether)
Velum	Fluopyram	Pyridinyl ethyl benzamide
Salibro	Fluazindolizine	Sulfonamide
Avicta, Tervigo	Abamectin	Macrocyclic lactones, metabolites of <i>Streptomyces avermectinius</i> (syn. <i>Streptomyces avermitilis</i>)
DiTera	Killed, dried fermentation product	Solids and solubles of <i>Myrothecium verrucaria</i> strain AARC-0255
Ecozin Plus 1.2% ME	Azadirachtin derived from neem	Botanical, extract from <i>Azadirachta indica</i>
MultiGuard Protect, Crop Guard	Furfural derived from sugarcane bagasse	Heterocyclic aldehyde, produced from various agricultural by-products
Nema-Q	Extract derived from soap bark tree	Botanical, extract from <i>Quillaja saponaria</i>
Sesamin EC, NeMaX	Sesame oil derived from sesame seeds	Botanical, extract from <i>Sesamum indicum</i>
BioNem, BioSafe; Votivo, Nortica	Live microorganisms	<i>Bacillus firmus</i> strain I-1582
Clariva	Live microorganisms	<i>Pasteuria nishizawae</i>
Klamic	Live microorganisms	<i>Pochonia chlamydosporia</i>
MeloCon, BioAct	Live microorganisms	<i>Purpureocillium lilacinum</i> strain 251

Notes: ^aThis list is for general information only. Active ingredients are often sold under various brand names, and many more products claim nematocidal activity. Inclusion on this list does not constitute an endorsement by the authors, nor is disapproval implied of products that are not mentioned. ^bMethyl isothiocyanate (MITC) releaser. ^cCombined with imidacloprid.

options are available in other crops and are currently being tested in sugarcane. These include fluensulfone, fluopyram and fluazindolizine. These new nematicides have a more favourable toxico-

logical profile than the older products and are nematocidal instead of nematostatic. They offer safer, effective chemical control options, with registration for use on multiple crops increasing.



Fig. 23.16. Soil fumigation under plastic mulch for *Rotylenchulus reniformis* control in pineapple in Hawaii. (Photograph courtesy of R.A. Sikora.)

Because of the differences in nematicide registration practices between countries, national registration offices should be contacted before use.

Planting material naturally free of infection

The production of healthy planting material is of utmost importance in nematode management because nematodes can be found in the seeds, tubers, corms or seedlings of many crops. The spread of nematodes can be prevented, or at least reduced, by the use of nematode-free seed/planting material and the use of nematode-free seedbeds or soils to produce clean seedlings for transplanting.

If a grower does not have a nematode-free planting area, nematode-free planting material can be selected, or the nematodes removed from the material before planting. Farmers producing their own seedlings will import fewer nematode problems than those purchasing seedlings from others, which may be nematode contaminated. Planting material that can be guaranteed free of root parasitic nematodes are certain crops propagated vegetatively from stem cuttings, such as sugarcane, sweet potato, cassava, black

pepper and planting stocks of many tree and vine crops. In certain states of Brazil, coffee seedlings are legally required to be certified free of root knot nematodes by a recognized nematology laboratory. Tissue culture production of plantlets, such as banana, is highly effective for producing nematode-free plants and is standard practice in commercial plantations worldwide (Fig. 23.17). Nematodes can produce damage symptoms (surface cracking, surface galls, watery lesions, necrotic spots, blackened roots, galls) in planting material such as bulbs, corms, tubers, seedlings and rootstocks, and farmers recognizing these symptoms as diseased or abnormal generally refrain from using the material for planting (Bridge, 1987, 1996).

Physical removal of tissues infected with nematodes

Physical removal of infected tissues is practised in several crops. The major nematode pests of bananas and plantains (*R. similis*, *Pratylenchus coffeae*, *Pratylenchus goodeyi*) can be removed from lightly infected planting material by cutting (paring) away roots, soil and purple-to-black nematode lesions and surrounding tissues from



Fig. 23.17. Nematode-free tissue culture banana plantlets growing in *Radopholus similis*-free greenhouse beds before transplanting into commercial plantations. (Photograph courtesy of L. Pocasangre.)

banana corms and suckers used for planting (Fig. 23.18). In yams (*Dioscorea* spp.), cutting out nematode dry rot lesions caused by *Scutellonema bradys* and *P. coffeae* from tubers can be effective in eliminating the nematodes from the seed pieces (Bridge and Page, 1982). A similar approach is used for taro.

Physical methods of nematode control in planting material

Hot water treatment of planting material can be very effective in controlling nematodes in seeds, bulbs, corms, tubers, rhizomes and rootstocks (Bridge, 1975; Maas, 1987; Gowen and Roberts, 2008). Accurate temperature baths and equipment to maintain the correct temperature, which is usually between 44°C to 55°C, are needed. Temperatures and duration required for the control of some nematodes are given in Table 23.9. The control of *R. similis* in banana corms with hot water baths has been recommended (Fig. 23.19), but has limited use, except by some small growers. A modification for resource-poor growers has been recommended to control migratory endoparasites in banana corms prior to planting in



Fig. 23.18. Physical removal of *Radopholus similis* from banana corms in Tonga by paring, with large dark spots being an indication of nematode infestation. (Photograph courtesy of P. Speijer and R.A. Sikora.)

India and East Africa (Prasad and Reddy, 1994; Mbwana *et al.*, 1998), including the use of boiling water for 30 s (Coyne *et al.*, 2010), and also in groundnuts in Africa (Bridge, 1975). Hot water treatment of garlic seed cloves with abamectin, bleach or other additives has proved effective for disinfecting *Ditylenchus dipsaci* (Gowen and Roberts, 2008); a treatment regime of abamectin

Table 23.9. Heat treatments used to control nematodes in planting material.

Nematode	Treatment
<i>Aphelenchoides besseyi</i>	Rice seed Cold soaking 18–25 h, 15 min, 51–53°C Cold soaking 3 h, 52–57°C No soaking, 55–61°C, 10–15 min
<i>Anguina tritici</i>	Wheat seed 4–6 h, 54°C, 10 min
<i>Ditylenchus dipsaci</i>	Onion bulbs 44–45°C for 3 h Garlic cloves 49°C for 20 min Shallot sets 44.5°C for 1–2 h
<i>Hirschmanniella miticausa</i>	Taro corms 50°C for 15 min
<i>Meloidogyne</i> spp.	Sweet potato 65 min at 47°C Yam 50–51°C, 30 min
<i>Meloidogyne incognita</i>	Sweet potato tubers 65 min at 47°C
<i>Meloidogyne javanica</i>	Potato tubers 2 h at 46–47.5°C
<i>Pratylenchus coffeae</i>	Yam tubers 46–52°C for 15–20 min
<i>Radopholus similis</i>	Banana corms 55°C for 15–25 min
<i>Scutellonema bradyi</i>	Yam tubers 50–55°C for 40 min

**Fig. 23.19.** Treatment of banana corms in a hot water bath to reduce *Radopholus similis* infection in Tonga. (Photograph courtesy of P. Speijer.)

at 10–20 ppm in an 18°C cool dip following a water hot dip of 49°C for 20 min is very effective, as is sodium hypochlorite at 1.1–3.3% aqueous solution as the 20-min hot dip.

Elimination of nematodes from seedbeds

Infested soils in seedbeds are often the main cause of nematodes being introduced into field soil on infected seedlings. Nematode-free soil for raising seedlings can be obtained from such localities as regularly flooded land. Soil taken from paddy rice production or from riverbanks is often free of nematodes. The soil should always be examined, however, to ensure it is free of major nematode

species, since the soil could be contaminated by runoff water from nearby fields. Soil infested with nematodes can be treated effectively by a range of physical or non-physical techniques (Tables 23.10 and 23.11).

At-planting Management

Date of planting

Planting date is a tool designed to reduce the impact caused by nematode penetration in the early growth stages by taking advantage of nematode inactivity. The fact that the minimum temperature required for *M. incognita* development

Table 23.10. Physical methods used to eliminate plant parasitic nematodes from infested soil.

Physical method of management	Description of method
Steam sterilization	Steam is passed under pressure into the soil under soil surface covers for 30 min for greenhouse high-value crops.
Application of boiling water	In Bolivia, farmers heat water on wood fires for seedbed treatment.
Heat sterilization	A soil sterilizer made from an oil drum and heated by a wood fire can be used to sterilize small amounts of moist soil.
Sun drying and heating	When steam rises, a lid is added and the fire removed for 1 h. Spreading soil, to a depth of 10 cm, on a soil-free surface exposed to the sun during the hot, dry season for a minimum of 2 weeks with regular turning will eradicate nematodes.
Turning soil to induce nematode desiccation	Nematodes can be killed by the lethal effects of heat from the sun and drying by turning the soil regularly at the end the growing season.
Surface burning of plant debris	Because heat has to penetrate deep into the soil to be effective, and substantial amounts of slow-burning output material are needed, this technique is not recommended, for environmental reasons.

Table 23.11. Non-physical methods for soil decontamination.

Non-physical method of management	Description of method
Annual or seasonal rotation of seedbed sites	Rotation of the seedbed areas each season or annually prevents the build-up of soil nematodes.
Keeping seedbeds free of weed hosts	Many weeds are hosts for the major nematodes that occur on transplanted crops, and their removal from the seedbed is important.
Floating seedling tray beds	Production of seedlings in floating trays over nematode-free water in vats will prevent nematode infection.
Chemical fumigation	Fumigation with nematicides has been used for many years to eradicate nematodes from infested soil. The recent removal of effective products from the market has affected their use.
Sealed-container solar heating	Soil is sealed in 5 kg polyethylene bags, which are placed in the sun on a concrete or black plastic surface for at least 2 weeks.
Biological enhancement	Biological enhancement of seedlings with beneficial microorganisms antagonistic to nematodes can increase resistance to nematodes.

in the root is significantly lower than the minimum 'activity threshold' of 18°C for *M. incognita* second-stage juveniles has been used to alter the date of planting for the control of root knot. Changing the normal date of planting to coincide with low soil temperature was considered an important control tactic on carrots (Roberts, 1987). This approach could also be used to limit nematode damage on vegetables in cool upland tropical regions. In Zimbabwe, the date of planting of tobacco is regulated to take advantage of cooler periods to reduce root knot infection. This is a technique that could have a major impact in other regions of the world (Shepherd and Barker, 1990).

The early planting of rice at cooler times of the year was effective in reducing *Aphelenchoides besseyi* on rice in the USA, and the early sowing of maize reduced damage caused by the cyst nematode *Punctodera chalcensis*. Planting date was used to avoid damage by *Globodera rostochiensis* that were still in diapause and unable to hatch and penetrate the sequentially planted potato crop in the Philippines (Sikora, 1984). Similar techniques have been developed for other nematodes on wheat and small grains (Johnson and Motsinger, 1990). Delayed planting of cotton also reduced root knot damage, and simultaneously that of the complex with fungal wilt (Jeffers and Roberts, 1993).

Bioenhancement

The biological enhancement of seeds and transplants with arbuscular mycorrhizal fungi (Fig. 23.20), mutualistic fungal endophytes, plant-health-promoting rhizosphere or mutualistic endophytic bacteria has been shown to increase plant resistance and/or tolerance to nematode infection during plant growth (Sikora and Hoffmann-Hergarten, 1993; Hallmann and Sikora, 1994; Sikora, 1997; Hallmann, 2001). Biological seed treatment of soybean, maize and cotton with the rhizobacterium *Bacillus firmus* product VOTiVO® and with the obligate bacterial parasite *Pasteuria nishizawae* product CLARIVA® to manage soybean cyst nematode and root knot is now used in IPM systems (Fig. 23.20).

Tomato and pepper transplant production substrate treated with different formulations of plant-growth-promoting rhizobacteria caused highly significant increases in tomato and pepper growth, vigour and survival in the field, with some formulations reducing the numbers of root knot galls on pepper (Kokalis-Burelle *et al.*, 2002). Endophytic bacteria have been shown to reduce root knot infection significantly and induce systemic resistance in tomato (Munif *et al.*, 2001).

The enhancement of plants with arbuscular mycorrhizal fungi (Fig. 23.21), apart from providing hosts with nutrients, reduces the penetration and development of nematodes in a range of crops, notably root knot nematodes, as well as other nematodes such as the burrowing nematode in banana, the yam nematode on yam and lesion nematodes on maize. Mycorrhizal



Fig. 23.20. *Pasteuria* spores attached to a second-stage juvenile of *Meloidogyne incognita*. (Photograph courtesy of K. Davis.)

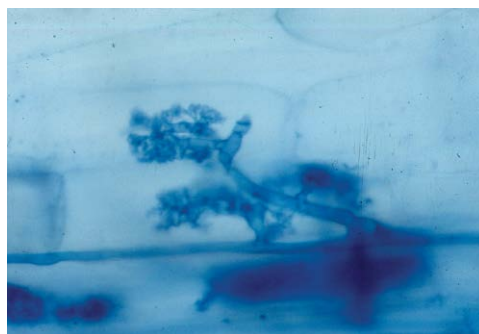


Fig. 23.21. Arbuscule of a mycorrhizal fungus in the roots of tomato, a fungus having both plant growth and health-promoting activity and used for the bioenhancement of transplants for root knot control. (Photograph courtesy of R.A. Sikora.)

inoculum is now commercially available for this purpose in many countries. Combining mycorrhizal fungi with plant-health-promoting rhizobacteria and mycorrhizal helper bacteria during seedling production and seedling growth has led to increased fungal colonization and root knot control in tomato seedlings (Reimann and Sikora, 2003). Endophytic fungi are prime antagonists for use in the biological enhancement of transplants for root knot control in vegetables (Hallmann and Sikora, 1994) and for the treatment of banana tissue culture plants for the management of burrowing and lesion nematodes (Niere *et al.*, 1998; Sikora, 2002; Sikora *et al.*, 2003; Zum Felde *et al.*, 2004; Waweru *et al.*, 2014).

The treatment of fumigated, biofumigated or solarized soil with biologically enhanced transplants would increase overall control, due to the lack of competitive microbial activity in this soil. To be effective, however, biological enhancement requires the availability of commercial biocontrol products, as is the case with mycorrhizal fungi, which can be used by small or large commercial nursery production units that supply bioenhanced seedlings to growers. In some crops for which large commercial companies produce healthy seedlings for their contract growers, the bioenhancement of planting material could lead to increased yield and reduced pesticide use.

Established Crop Management

The management of nematode infestations after planting is an important tool for many crops, in particular for perennial crops such as fruit and tree crops and spices. The most important plant management tool is plant resistance to nematode attack for crop–nematode combinations where effective resistance has been bred into cultivars or rootstocks. However, where resistance is not available, other tools have been developed that ensure good yield even in the presence of nematodes in the rhizosphere. There are only a few methodologies that can be used curatively to reduce or inhibit nematode damage once the crop is in the field. However, in some crops they are the mainstay of nematode management strategies; for example, on banana, citrus, fruit and tree crops.

Host resistance and tolerance

Host resistance, if available in a high-yielding cultivar, can provide the foundation on which other management tactics can be integrated. Resistance is typically defined as a plant’s ability to inhibit nematode reproduction relative to that on a susceptible genotype (Cook and Evans, 1987; Trudgill, 1991; Roberts, 2002; Williamson and Roberts, 2009). Thus, resistance is distinct from the effects of nematode parasitism on plant growth and yield. Tolerance and intolerance are most often used to describe a plant’s response to parasitism, with a tolerant plant experiencing less yield suppression than an intolerant plant at similar levels of parasitism (Cook and Evans, 1987; Trudgill, 1991; Roberts, 2002). In many cases, resistance in plants affords protection against nematode feeding

and plant injury and thus they perform as tolerant plants, but in some plants tolerance is inherited independently from resistance (see Trudgill, 1991; Roberts, 2002). Some resistant crop genotypes are known to be relatively intolerant of nematode parasitism, (Johnson *et al.*, 1989), for example, sweet potato resistant to root knot nematode (Roberts and Scheuerman, 1984), while some susceptible crop cultivars can tolerate high nematode densities, such as maize, grasses and other plants with rapid root regeneration potential (Roberts, 2002). Also, the level of resistance and the level of tolerance both fall somewhere on a continuum, depending on the quantitative nature of the trait determinants. The four possible combinations of these two distinct traits for any given crop are given in Table 23.12.

Often, low to moderate levels of resistance are referred to as tolerance, and equated with intolerance. However, both resistance and tolerance are traits that can only be assessed relative to the performance of another genotype of the same species, typically a known susceptible, intolerant genotype. An example of the desired performance comparisons for assessing yields of advanced lines or new cultivars is provided in Roberts and May (1986) for processing tomatoes and the *Mi-1* gene for root knot nematode resistance. Resistant and susceptible cultivars were evaluated in a four-way comparison in *M. incognita* infested versus pre-plant fumigated (low initial density) test plots. Resistant and susceptible tomato yields were similar following fumigation, and the yield in infested versus fumigated plots was not significantly different for resistant cultivars, whereas susceptible cultivars had, on average, 50% less yield on infested plots (Roberts and May, 1986). These results confirmed the effectiveness of the *Mi-1* resistance and also confirmed that resistant

Table 23.12. Possible combinations of resistance, susceptibility, tolerance and intolerance in a crop genotype with respect to nematode reproduction and plant response to nematode parasitism. (From Trudgill, 1991.)

Nematode reproduction	Host growth	
	Good	Poor
Good	Tolerant/susceptible	Intolerant/susceptible
Poor	Tolerant/resistant	Intolerant/resistant

cultivars performed as well as susceptible ones in the absence of nematode damage. The additional benefits of nematode resistance, which should be evaluated and utilized, are in the management of nematode–disease complexes, for example in root knot–*Fusarium* wilt interaction, where nematode resistance can reduce the severity of fungal wilt infection, for example in cotton and cowpea (Roberts *et al.*, 1995).

In subtropical and tropical environments, where most nematode parasites complete multiple generations on annual crops, the reduction in total parasitism due to reduced nematode reproduction typically results in increased crop yields, and intolerant resistant genotypes will likely appear tolerant. In perennial crops, the long-term cumulative effects of reduced nematode reproduction are even greater than in annual crops. An understanding of the effects of resistance and tolerance traits on crop yield and nematode population densities is important to advance our knowledge of plant nematode interactions and to guide plant breeding programmes and nematode management decisions.

Because resistance typically leads to improved yields in fields infested with nematode population densities that exceed the damage threshold, resistance protects the genetic yield potential of the crop. This is the most important benefit to be derived from the use of resistant cultivars or rootstocks and should be the characteristic that is most appealing for farmers to adopt resistance. In some cases where the nematode density is less than the damage threshold, a resistant cultivar may have a lower yield potential than that of a high-yielding susceptible cultivar. No data are available that show a direct effect of resistance genes on reduced yield potentials. Such apparent negative effects of resistance on yield potential are generally associated with linkage drag, whereby genes with negative effects on yield potential are linked to resistance loci. Historically, this has been the case because of the challenges in breeding programmes to introgress resistance traits into cultivars that, in the absence of nematode damage, yield as well as the best current elite cultivars. Better efforts to test resistant advanced breeding lines in yield trials with and without nematode infestation and under different soils and environments have improved the yield potential of resistant

cultivar releases. In the past decade, breeding for nematode resistance and other traits has advanced considerably through major developments in molecular marker technologies. These allow for accurate genetic mapping of target trait determinants such as quantitative trait locus (QTL) mapping analysis and marker-assisted selection (MAS), using high-throughput genotyping protocols, such as single nucleotide polymorphism (SNP) marker platforms and genotyping-by-sequencing approaches. The technologies enable both foreground and background genomic selection, allowing for the inclusion of the resistance trait determinants together with recovery of the elite cultivar background; Huynh *et al.* (2016) provide an example with root knot nematode resistance in the grain legume, cowpea.

Efforts to develop soybean cultivars with resistance to *H. glycines* have been in progress for more than 40 years, thus one would expect that resistance would be available in cultivars with the highest yield potentials. Currently, almost all soybean cultivars grown in the USA have resistance to one or more races or HG types of *H. glycines* (Mitchum, 2016). For root knot nematodes, almost all current cultivars of processing tomato grown in the USA, primarily in California, have root knot resistance conferred by gene *Mi-1*. Similarly, nearly all modern wheat cultivars contain multiple genes for resistance to fungi and viruses and there is no evidence that these multiple-resistance genes have a negative effect on yield. These examples show that modern breeding for nematode and other pathogen resistance has, to some degree, overcome the earlier issues of combining resistance with high yield performance.

Differential effects of resistance and tolerance on nematode population densities result in different effects on the productivity of cropping systems involving multiple crops with a range of tolerances and levels of resistance. As demonstrated by Ogallo *et al.* (1999), lima beans susceptible to *M. incognita* can be grown successfully following two plantings of a root knot-resistant cotton cultivar, but experience heavy yield losses when grown following two plantings of a susceptible cultivar. In similar studies, Roberts *et al.* (2005) showed that cowpea cultivars carrying the *Rk* gene for resistance to *M. incognita* were highly effective in protecting a following susceptible

tomato in a legume–tomato rotation system. Although a susceptible/tolerant crop will have greater yield than a susceptible/intolerant crop in a nematode-infested field, because of the relatively high level of nematode reproduction on the susceptible/tolerant cultivar, the potential for yield suppression of an intolerant crop following the susceptible/tolerant crop will be similar to that when following a susceptible/intolerant crop. Another possible situation is that crop genotypes with tolerance or low levels of partial resistance may actually result in a greater hazard to a subsequent susceptible crop than when the first crop in the sequence is susceptible and intolerant. The enhanced host root growth because of tolerance or partial resistance provides a larger resource for nematode population increase, resulting in high final population densities, which in turn provides high initial densities to the following crop. This phenomenon was shown to occur on soybean cultivars with partial resistance to root knot nematode (Niblack *et al.*, 1986).

Resistance, when available, is not a universal solution to nematode management. As resistance is highly specific, being effective against only a single species, or even only one race or biotype of a species, it will not control other potential nematode pests in fields with a polyspecific community. This can be a major limitation to the use of resistance, but is not a limitation in cases where the crop only has one major nematode pest species or where a field is infested with only one major pest species. Genetic tolerance may be less specific than resistance and may work against several nematode species, but this hypothesis has not been tested. In crops with partial resistance to one or more nematode species, some yield loss is to be expected at high initial nematode densities, such that resistance must be used in combination with other management tactics to achieve the maximum yield potential. In fact, partial resistance may make other management tactics more effective through additive controls. This is analogous to cases where partial resistance to foliar fungal pathogens, which is of limited value as a sole management tactic, has great value in an integrated programme and permits a reduction in the reliance on fungicides (Maytac and Bailey, 1988).

Resistance may lack durability because repeated use of single resistance genes often leads

to a shift in the virulence characteristics of the nematode population, such that with time a specific resistance gene is no longer effective. This has been demonstrated with *Globodera* species on potato (Turner, 1990), *H. glycines* on soybean (Gardner *et al.*, 2017), and for root knot nematodes where virulence in *M. incognita* and *M. javanica* to the *Mi-1* gene in tomato has been identified (Kaloshian *et al.*, 1996; Orenat *et al.*, 2001). The dramatic spread of *H. glycines* in the USA over 60 years (Tylka and Marett, 2014), coupled with the intensive use of resistant soybean cultivars from a limited source of major gene resistance, has resulted in the widespread failure of resistance due to genetic selection for virulence (Mitchum, 2016). In the case of *Mi-1* major gene resistance to root knot in tomato, the extensive use of resistant cultivars without integration with other control tactics has led to several dozen fields with virulent *M. incognita* or *M. javanica* populations, rendering the resistance ineffective. In California processing tomatoes, the first cultivar with resistance was released in 1980, and it took about 25 years for the multiple-field breakdown of resistance to become noticeable. Alternative genes for resistance are available in these crops but require concerted efforts to breed them into elite cultivars.

However, if the nematode population in a given field or region lacks the appropriate diversity with respect to virulence, then there may not be selection for virulence with repeated use of a given resistance gene. This appears to be the case for the *H1* gene for resistance to *G. rostochiensis* in some regions (Trudgill and Parrott, 1972). Similarly, repeated use of resistance may cause a shift in the species present in a field, with species against which the resistance is not effective becoming dominant. This has been documented for tobacco, where the increased use of resistance to *M. incognita* led to an increase in the frequency of *M. javanica* against which the resistance was not effective, and in potato where the use of resistance to *G. rostochiensis* led to an increased incidence of *Globodera pallida* (Trudgill, 1991; Roberts, 1995). In some perennial tree crops, such as *Prunus* spp. and citrus, rootstock resistance has remained effective, and hence durable, for many decades; for example, in Nemaguard rootstock resistant to root knot, or trifoliolate orange rootstock resistant to the citrus

nematode (*Tylenchulus semipenetrans*). An additional complication for a few resistance genes, most notably the tomato *Mi-1* gene, is their sensitivity to and ineffectiveness at high soil temperature (for *Mi-1* > 28°C) (Roberts, 1995), which limits their use in tropical climates. Resistance is currently available to several nematodes in a relatively limited number of crops, primarily to species of the more specialized endoparasitic genera (Table 23.13), reflecting a great need for the development of resistance in additional crops and against a broader range of nematodes.

Post-planting nematicide treatment

There are a number of systemic non-fumigant nematicides listed in Table 23.8 that are used to control nematodes in the standing crop in the field. Because many of these compounds are not phytotoxic, they can be applied directly to the growing

plant to inhibit nematode development in the root tissue. When applied after plant establishment, these systemic compounds inhibit nematode development in the root for weeks after application and reduce additional penetration of the root. This dual action can lead to significant root growth and plant resilience to follow-up infection.

In banana production, where *R. similis* is a major problem, these systemic nematicides are often applied two to three times in each growing cycle. Similar approaches are used on other perennial crops, for example, for many tree and vine crops (see Chapters 12, 13, 16, 17 and 18, this volume). In groundnut, besides pre-plant treatment, established plants are often treated with a second application of nematicides before canopy filling to prevent damage to the pods later in the season (see Chapter 11, this volume). Application through drip irrigation over an extended period of time has also been effective in limiting root knot in vegetable production. Soil treatment with the egg pathogenic facultative

Table 23.13. A partial list of food and fibre crops for which high-yielding cultivars with resistance to one or more nematode species are available or in advanced breeding lines.

Crop	Nematode species
Banana	<i>Radopholus similis</i> , <i>Pratylenchus coffeae</i>
Barley	<i>Heterodera avenae</i>
Bean, common	<i>Meloidogyne incognita</i>
Bean, lima	<i>M. incognita</i> , <i>Meloidogyne javanica</i>
Citrus	<i>Tylenchulus semipenetrans</i>
Clover	<i>Ditylenchus dipsaci</i>
Coffee	<i>Meloidogyne arabicida</i> , <i>Meloidogyne exigua</i> , <i>M. incognita</i> , <i>Meloidogyne paranaensis</i>
Cotton	<i>M. incognita</i>
Cowpea	<i>Meloidogyne arenaria</i> , <i>Meloidogyne hapla</i> , <i>M. incognita</i> , <i>M. javanica</i>
Grape	<i>M. arenaria</i> , <i>M. incognita</i> , <i>M. javanica</i>
Groundnut	<i>M. arenaria</i> , <i>M. javanica</i>
Lucerne	<i>D. dipsaci</i> , <i>M. hapla</i>
Maize	<i>Pratylenchus hexincisus</i>
Peach, plum (<i>Prunus</i> rootstocks)	<i>M. arenaria</i> , <i>M. incognita</i> , <i>M. javanica</i>
Pepper, bell, chilli	<i>M. arenaria</i> , <i>M. incognita</i> , <i>M. javanica</i>
Potato	<i>Globodera pallida</i> , <i>Globodera rostochiensis</i>
Oat	<i>D. dipsaci</i> , <i>H. avenae</i>
Rice	<i>Aphelenchoides besseyi</i> , <i>Ditylenchus angustus</i>
Soybean	<i>Heterodera glycines</i> , <i>M. arenaria</i> , <i>M. incognita</i> , <i>M. javanica</i> , <i>Rotylenchulus reniformis</i>
Sweet potato	<i>M. arenaria</i> , <i>M. incognita</i> , <i>M. javanica</i> , <i>R. reniformis</i>
Tobacco	<i>Globodera tabacum</i> , <i>M. incognita</i>
Wheat	<i>H. avenae</i> , <i>Pratylenchus neglectus</i> , <i>Pratylenchus thornei</i>

fungus *Purpureocillium lilacinum* (Bioact®) via drip irrigation at regular intervals during crop production is commercially available for use on a wide range of crops for root knot nematode management in the USA.

Caution, however, should be taken to avoid the overuse of non-fumigant nematicides as a management tool, since in some cases it can lead to microbial breakdown of the compounds and loss of nematicidal efficacy (Stirling *et al.*, 1992; Ou *et al.*, 1994; Cabrera *et al.*, 2009). Therefore, proper application management and rotation of compounds is necessary for prolonged efficacy.

Grafting

One of the most effective and innovative techniques developed for nematode management is the grafting of commercially valuable crop cultivars on to nematode- and disease-resistant rootstocks (Fig. 23.22). Although grafting has been

practised since the 1920s in Japan and Korea, it has only recently become highly regarded in protected cultivation for disease and nematode control. The grafting technique has been used largely in the horticulture industry to manage soil-borne diseases and pests, such as root knot nematodes, fungi (i.e. corky root disease – *Pyrenochaeta lycopersici*) or virus. In Japan, 59% of the cucumber, tomato, aubergine, watermelon and melon grown in protected cultivation are tube grafted on to rootstocks of various types. Depending on the rootstock, the technique can lead to increased plant vigour and tolerance or resistance to nematodes and diseases. Depending on the price of production, grafting can be very effective in both field and protected cultivation of vegetables. Grafting susceptible scions on to nematode-resistant rootstocks has been used very effectively for the control of root knot or lesion nematodes for many years in fruit and nut crops, e.g. citrus, grapes, *Prunus* (peaches, plums, almonds) and walnuts (Nyczepir and Halbrecht, 1993; Saucet *et al.*, 2016).

Species of *Solanum* have been shown to have a high level of resistance to *M. incognita* and



Fig. 23.22. Grafting a susceptible tomato shoot on to the roots of a resistant tomato cultivar. Upper left: cutting stem at 45-degree angle; upper right: aligning tube sleeve treated with growth hormone; lower left: fusing shoot to root; lower right: resulting grafted tomato seedling. (Photograph courtesy of D. Kenny, AVRDC, Taiwan.)

M. arenaria, but they are poor hosts for *M. javanica* and have been used successfully as rootstocks. Of seven wild species of *Solanum* tested, four accessions were resistant to *M. incognita*, i.e. *Solanum sisymbriifolium*, *Solanum torvum*, *Solanum toxicarium* (Mian *et al.*, 1995) and *Solanum huaylasense* (Cortada *et al.*, 2010), and also reduced bacterial wilt. Granges and Leger (1996) showed that when susceptible tomatoes were grafted on to rootstocks having resistance to species of *Meloidogyne* and various root pathogens, yield increased 50% and 30% at the beginning and end of harvest when compared to the non-grafted plants, respectively. Grafting has been tested in many countries and is widely used for root knot and bacterial wilt control in tomato. In tomato, susceptible shoots are grafted on to resistant rootstocks (López-Pérez *et al.*, 2006). Similarly, resistant *Cucumis metuliferus* rootstocks can be used to support susceptible scions of melons for root knot control (Siguenza *et al.*, 2005).

For the control of root knot nematodes, solanaceous crops (i.e. tomatoes and aubergines) and cucurbits (i.e. watermelon) are often grafted on to commercial *Mi*-resistant tomato hybrid rootstocks (*Solanum* spp. $\times$ *Solanum lycopersicum*). Nevertheless, studies show that the efficacy to control root knot nematodes varies among them, as some of these present both inter- and intraspecific variability to *M. incognita*, *M. javanica* and *M. arenaria* (Cortada *et al.*, 2008 and 2009); the phenotypic response of the *Mi*-resistant tomato rootstocks can be extraordinarily variable, ranging from highly resistant (Pf/P < 0.1) to susceptible (Cortada *et al.*, 2008, Graf *et al.*, 2001; López-Pérez *et al.*, 2006). This has been attributed to the presence of multiple *Mi*-homologues in the genetic background of these hybrids (Cortada *et al.*, 2012) that are different from the *Mi-1.2* gene from *Solanum peruvianum*, which confers resistance to the *Meloidogyne* spp. listed above in the traditional *Mi*-resistant tomato cultivars (*S. peruvianum* $\times$ *S. lycopersicum*) (Kaloshian *et al.*, 1998). This complex genetic background of the *Mi*-resistant hybrid rootstocks has been observed in situations where the selection of *Mi*-virulent populations of root knot nematodes occurred after only two consecutive cropping seasons (Verdejo-Lucas *et al.*, 2009), or when certain *Mi*-resistant rootstocks showed a robust resistance response to root knot nematodes when subjected to

intermittent temperature peaks above 28°C, where *Mi-1.2*-mediated resistance remained effective (Verdejo-Lucas *et al.*, 2012, 2013).

Despite the high variability observed among the existing commercial *Mi*-resistant tomato hybrid rootstocks, some of them have shown a remarkable ability to reduce the infestations of root knot nematodes in the soil and increase crops' productivity. Therefore, these should be considered as an effective and profitable tool that could help farmers to minimize yield losses caused by root knot nematodes and other soil-borne diseases, and to mitigate the negative impact of the agronomic stress associated with intensive farming practices. Nevertheless, the selection and management of the commercial *Mi*-resistant tomato hybrid rootstocks must be done judiciously: it is recommended to rotate different *Mi*-tomato hybrid rootstocks and *Mi*-cultivars with root knot nematode-susceptible varieties (Talavera *et al.*, 2009), with other crops, and/or fallow, to prevent the appearance of *Mi*-virulent populations that would invalidate the use of natural resistance against root knot nematodes.

Improved crop husbandry

Proper fertilizer use and proper moisture levels help plants to compensate for nematode damage. This is probably true when a plant is attacked by sub-threshold densities as opposed to high densities. Improved fertilizer application, especially nitrogen, has been shown to increase yield in a number of crops infected with nematodes (Brown, 1987). Proper plant management after pre-plant nematode control, obviously, will ensure a stronger root system and thereby reduce the effects of nematode penetration on the early stages of plant growth.

Postharvest Management

Root destruction

Given that nematodes can survive and reproduce on viable root tissue remaining in the soil after harvest, roots should be eliminated by uprooting and destruction whenever possible. The

spread of the nematode to the follow-up crop will be retarded and the overall population density reduced. It has been estimated that when soil temperatures are high, each month that the root system survives causes a tenfold increase in root knot nematode densities. Root knot, for example, can even survive and reproduce in excavated roots and tubers over many weeks in such crops as tomato, potato, banana and pepper, and even in small pieces of sweet potato tubers. Root removal and the burning of tobacco roots, as well as ploughing the field after harvest to encourage root degradation, will reduce the impact of root knot nematodes on subsequent crops (Shepherd and Barker, 1990). LaMondia (2008) demonstrated that destroying the stalks and root systems remaining after harvesting stalk-cut, broadleaf, cigar wrapper tobacco prevented the development of two generations of *Globodera tabacum tabacum*, resulting in lower tobacco cyst nematode densities than without removal. The use of herbicide injection into tree and banana roots at the termination of a perennial crop planting can be used to prevent feeding of lesion, burrowing and root knot nematodes that otherwise survive in old roots and present infection potential for the next crop (Chabrier and Queneherve, 2003). In many eastern and western African countries, the removal of roots attached to the shoots of short-cycle, sequentially cropped black nightshade, *Solanum nigrum*, eliminates root knot by trapping females before the initiation of egg laying (R.A. Sikora, unpublished data) (Fig. 23.23).

Time of harvesting

The use of day degrees or the temperature sum needed to complete a life cycle can be used to time the harvest to trap the last life cycle of a nematode and thereby reduce nematode densities. This has been demonstrated for cyst nematodes that have a long duration life cycle; for example, the sugarbeet cyst nematode *H. schachtii* and the potato cyst nematode *G. rostochiensis*. The early harvesting of sugarbeet in the hot soils of the Imperial Valley of California can prevent the completion of a third, fourth or even fifth generation of *H. schachtii* (Thomason and Fife, 1962). The early harvesting of carrot



Fig. 23.23. Harvesting African nightshade, *Solanum nigrum*, with roots attached after 3–4 weeks growth, trapping root knot before egg laying. (Photograph courtesy of R.A. Sikora, Benin.)

can likewise limit root knot nematode multiplication and final population densities (Roberts, 1987). Using short-maturity groupings of crops, such as soybean, can also limit nematode build-up (Koenning *et al.*, 1993).

Integrated Nematode Management Strategies

The development of nematode integrated management programmes in many subtropical and tropical production systems requires not only careful analysis of the impact of the control technology on the nematode population within often complex multi-crop production systems but also determination of cost–benefit ratios for grower acceptance, as outlined in this chapter. Analysis of both the temporal and spatial target effects of different control tactics in a cropping

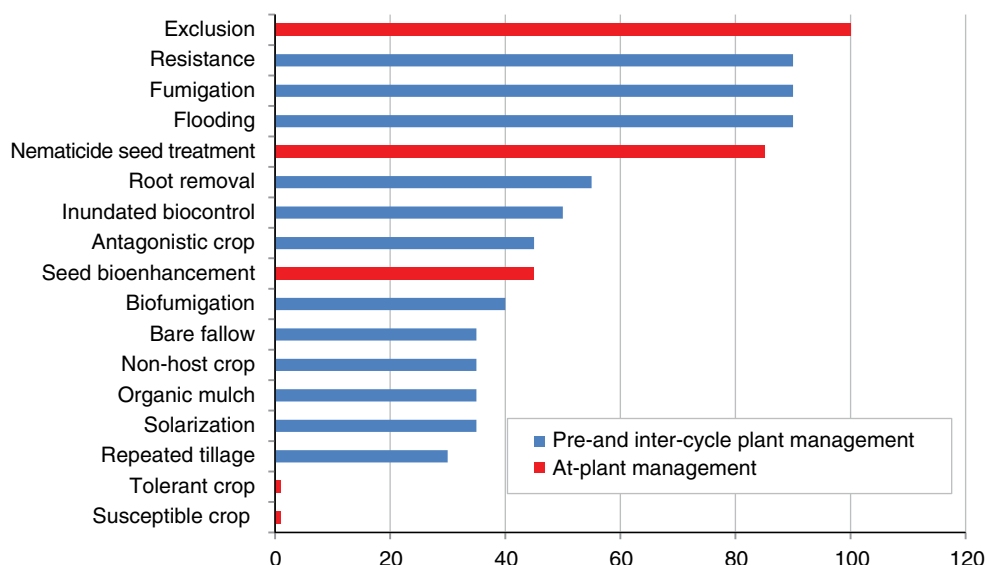


Fig 23.24. Estimated impact of pre-, inter-cycle and at-plant management practices on per cent reduction of root knot nematode early root penetration.

system must be carried out to optimize the integrated management system (Roberts, 1993). We have discussed the logical placement of the broad number of management tools in the growing cycle that are available to nematologists, as well as their potential and limitations for nematode management. In Fig. 23.24, an attempt is made to give an estimate of the maximum impact these management tools can have on a nematode population in the soil after treatment. The estimates of the levels of control have been extracted from chapters dealing with control in this volume and from past research (Brown and Kerry, 1987; Luc *et al.*, 1990; Evans *et al.*, 1993; Barker *et al.*, 1998). The estimates are provided as a guideline for the development of new approaches to nematode management.

The level of control will be influenced by: environmental factors, such as soil type, moisture

and temperature, the crop and the nematode targeted, as well as the crop management programme being used. Proper application of the technology is of utmost importance. Each control method listed has advantages and disadvantages that need to be understood in the development of a new management programme, often over many growing cycles and seasons. In the chapters in this book, integrated control options have been outlined in detail for all crops and nematodes of economic importance in the tropics and subtropics. The advantages and disadvantages of these methods have been discussed in this chapter, and more so in the chapters in this volume. Nematode management in the future will require a sustainable systems approach based on the logical use of effective control methodologies in combinations that are economically acceptable and practical for use by the growers in the subtropics and tropics.

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Appendix – Plant Parasitic Nematode Genera and Species Cited*

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All genera and species of plant parasitic nematodes cited in the book are listed alphabetically. Generic names are in bold font. The genera and species are followed by their 'authorities', i.e. the name(s) of the author(s) of the original description, in some cases followed by the name(s) of the author(s) who have published the more recent valid taxonomic name, that is, by placing the species in another genus. In such cases, the original authorities are placed in parentheses. Both authorities are followed by their respective year of publication. In the criconematids, some confusion may be experienced in the apparently arbitrary use of the names *Criconemella*, *Criconemoides*, *Macroposthonia* and *Mesocriconema*. The oldest generic name, *Macroposthonia*, has been suppressed by the ICZN, with *Criconemoides*, the next available name, now being recognized as valid. However, *Criconemoides sensu lato* is often split into *Criconemoides sensu stricto* and *Mesocriconema*. The latter, which contains the major plant parasitic species, is a complex and speciose assemblage that differs from *Criconemoides sensu stricto* by several small morphological characters and which appears to clade separately using molecular techniques. It is, perhaps, simplest to remember that all *Mesocriconema* species can be regarded as belonging to *Criconemoides sensu lato*, but that not all *Criconemoides*

species can be attributed to *Mesocriconema*. In the list that follows, both generic names are used and the species cross-referenced where necessary.

The most common synonyms are also listed alphabetically as 'cf.' and prefaced by '=' below the currently valid name.

For each genus, the group to which it is currently assigned is indicated in square brackets, as follows:

- Tyl. Tylenchina (Tylenchomorpha)
- A. Aphelenchoididae (Tylenchomorpha)
- L. Longidoridae (Dorylaimina)
- P. Panagrolaimidae (Rhabditida)
- T. Tylencholaimidae (Dorylaimina)
- Tri. Trichodoridae (Diphtherophorina)

List of Genera and Species

Achlysiella Hunt, Bridge & Machon, 1989 [Tyl., Pratylenchidae]

Achlysiella williamsi (Siddiqi, 1964) Hunt, Bridge & Machon, 1989

= *Radopholus williamsi* Siddiqi, 1964

Afenestrata Baldwin & Bell, 1985 [Tyl., Heteroderidae]

Afenestrata africana

cf. *Heterodera africana*

* A revision of the Appendix by M. Luc and D.J. Hunt in the second edition.

Afrinacf. *Subanguina**Afrina wevelli*cf. *Subanguina wevelli***Allotrichodorus** Rodriguez-M, Sher & Siddiqi, 1978 [Tri.]*Allotrichodorus brasiliense* Rashid, De Waele & Coomans, 1986*Allotrichodorus campanulatus* Rodriguez-M, Sher & Siddiqi, 1978*Allotrichodorus sharmae* Rashid, De Waele & Coomans, 1986*Allotrichodorus westindicus*cf. *Ecuadorus westindicus***Amplimerlinius** Siddiqi, 1976 [Tyl., Belonolaimidae]**Anguina** Scopoli, 1777 [Tyl., Anguinidae]*Anguina agrostis* (Steinbuch, 1799) Filipjev, 1936*Anguina tritici* (Steinbuch, 1799) Chitwood, 1935**Aorolaimus** Sher, 1963 [Tyl., Hoplolaimidae]= *Peltamigratus* Sher, 1964*Aorolaimus banoae* (Rashid, Geraert & Sharma, 1987) Baujard, Castillo, Doucet, Martiny, Mounport & N'Diaye, 1991= *Peltamigratus banoae* Rashid, Geraert & Sharma, 1987*Aorolaimus holdemani* (Sher, 1964) Fortuner, 1987= *Peltamigratus holdemani* Sher, 1964*Aorolaimus levicaudatus* (Bittencourt & Huang, 1986) Baujard, Castillo, Doucet, Martiny, Mounport & N'Diaye, 1991= *Peltamigratus levicaudatus* Bittencourt & Huang, 1986*Aorolaimus luci* (Sher, 1964) Fortuner, 1987= *Peltamigratus luci* Sher, 1964*Aorolaimus nigeriensis* (Sher, 1964) Fortuner, 1987= *Peltamigratus nigeriensis* Sher, 1964*Aorolaimus vigiae* (Rashid, Geraert & Sharma, 1987) Baujard, Castillo, Doucet, Martiny, Mounport & N'Diaye, 1991= *Peltamigratus vigiae* Rashid, Geraert & Sharma, 1987**Aphasmatylenchus** Sher, 1965 [Tyl., Hoplolaimidae]*Aphasmatylenchus liberiensis* Baujard, Vovlas, Mounport & Martiny, 1998*Aphasmatylenchus nigeriensis* Sher, 1965*Aphasmatylenchus straturatus* Germani, 1970**Aphelenchoides** Fischer, 1894 [A.]*Aphelenchoides aligarhiensis* Siddiqi, Husain & Khan, 1967*Aphelenchoides arachidis* Bos, 1977*Aphelenchoides besseyi* Christie, 1942= *Aphelenchoides oryzae* Yokoo, 1948*Aphelenchoides bicaudatus* (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941*Aphelenchoides ensete* Swart, Bogale & Tiedt, 2000*Aphelenchoides fragariae* (Ritzema-Bos, 1890) Christie, 1932*Aphelenchoides ritzemabosi* (Schwartz, 1911) Steiner & Buhrer, 1932**Aphelenchus** Bastian, 1865 [A., Aphelenchidae]*Aphelenchus avenae* Bastian, 1865**Apratylenchus** Trinh, Waeyenberge, Nguyen, Baldwin, Karssen & Moens, 2009 [Tyl., Pratylenchidae]*Apratylenchus binhi* Trinh, Waeyenberge, Nguyen, Baldwin, Karssen & Moens, 2009*Apratylenchus vietnamensis* Trinh, Waeyenberge, Nguyen, Baldwin, Karssen & Moens, 2009**Atalodera** Wouts & Sher, 1971 [Tyl., Heteroderidae]= *Thecavermiculatus* Robbins, 1978*Atalodera andina* (Golden, Franco, Jatala & Astogaza, 1983) de Souza & Huang, 1994= *Thecavermiculatus andinus* Golden, Franco, Jatala & Astogaza, 1983*Basirolaimus*cf. *Hoplolaimus***Belonolaimus** Steiner, 1949 [Tyl., Belonolaimidae]= *Ibipora* Monteiro & Lordello, 1977*Belonolaimus euthychilus* Rau, 1963*Belonolaimus gracilis* Steiner, 1949*Belonolaimus longicaudatus* Rau, 1958*Belonolaimus maritimus* Rau, 1963*Belonolaimus nortoni* Rau, 1963*Bitylenchus dubius*cf. *Tylenchorhynchus dubius***Bursaphelenchus** Fuchs, 1937 [A.]= *Rhadinaphelenchus* Goodey, 1960*Bursaphelenchus cocophilus* (Cobb, 1919) Baujard, 1989= *Rhadinaphelenchus cocophilus* (Cobb, 1919) Goodey, 1960*Bursaphelenchus mucronatus* Mamiya & Enda, 1979*Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970

- Cacopaurus** Thorne, 1943 [Tyl., Tylenchulidae]
- Cactodera** Krall & Krall, 1978 [Tyl., Heteroderidae]
Cactodera amaranthi (Stoyanov, 1972) Krall & Krall, 1978
- Caloosia** Siddiqi & Goodey, 1964 [Tyl., Cricone-matidae]
Caloosia exilis Mathur, Khan, Nand & Prasad, 1969
Caloosia heterocephala
 cf. *Caloosia paxi*
Caloosia nudata (Colbran, 1963) Brzeski, 1974
 = *Hemicycliophora nudata* Colbran, 1963
Caloosia paradoxa (Luc, 1958) Brzeski, 1974
 = *Hemicycliophora paradoxa* Luc, 1958
Caloosia paxi Mathur, Khan, Nand & Prasad, 1969
 = *Caloosia heterocephala* Rao & Mohandas, 1976
- Cephalenchus** Goodey, 1962 [Tyl., Tylenchidae]
Cephalenchus emarginatus (Cobb, 1893) Geraert, 1968
Cephalenchus hexalineatus (Geraert, 1962) Geraert & Goodey, 1964
- Coslenchus** Siddiqi, 1978 [Tyl., Tylenchidae]
Coslenchus pastor Andr ssy, 1982
- Criconema** Hofm nner & Menzel, 1914 [Tyl., Cricone-matidae]
Criconema braziliense (Raski & Pinochet, 1976) Raski & Luc, 1985
 = *Mesocriconema braziliense* Raski & Pinochet, 1976
Criconema cardamomi (Khan & Nanjappa, 1972) Raski & Luc, 1985
Criconema coorgi (Khan & Nanjappa, 1972) Raski & Luc, 1985
Criconema corbetti (De Grisse, 1967) Raski & Luc, 1985
 = *Nothocriconema corbetti* De Grisse, 1967
Criconema crassianulatum (de Guiran, 1963) Raski & Luc, 1985
Criconema demani Micoletzky, 1925
Criconema jaejuense (Choi & Geraert, 1975) Raski & Luc, 1985
 = *Nothocriconema jaejuense* Choi & Geraert, 1975
- Criconemella** [Tyl., Cricone-matidae]
 cf. *Criconemoides* or *Mesocriconema*
- Criconemoides** Taylor, 1936 [Tyl., Cricone-matidae]
 = *Macroposthonia* de Man, 1880 (= *gen. dub.*)
 = *Criconemella* De Grisse & Loof, 1965
 [= *Mesocriconema* Andr ssy, 1965 in some taxonomic schemes]
Criconemoides annulatus Cobb in Taylor, 1936 *nec*
Macroposthonia annulata de Man, 1880 [*sp. inq.*]
Criconemoides axestis Fassuliotis & Williamson, 1959
 = *Criconemella axestis* (Fassuliotis & Williamson, 1959) Luc & Raski, 1981
 = *Mesocriconema axeste* (Fassuliotis & Williamson, 1959) Loof & De Grisse, 1989
Criconemoides brevistylus Singh & Khera, 1976
 = *Mesocriconema brevistylus* (Singh & Khera, 1976) Loof & De Grisse, 1989
Criconemoides curvatus Raski, 1952
 = *Criconemella curvata* (Raski, 1952) Luc & Raski, 1981
 = *Mesocriconema curvatum* (Raski, 1952) Loof & De Grisse, 1989
 = *Criconemoides tescorum* de Guiran, 1963
 = *Macroposthonia tescora* (de Guiran, 1963) De Grisse & Loof, 1965
 = *Mesocriconema tescorum* (de Guiran, 1963) Loof & De Grisse, 1989
Criconemoides denoudeni Heyns, 1962
 = *Mesocriconema denoudeni* (Heyns, 1962) Loof & De Grisse, 1989
Criconemoides dherdei (De Grisse, 1967) Luc, 1970
 = *Mesocriconema dherdei* (De Grisse, 1967) Loof & De Grisse, 1989
Criconemoides ferniae Luc, 1959
 = *Criconemella ferniae* (Luc, 1959) Raski & Luc, 1981
 = *Mesocriconema ferniae* (Luc, 1959) Loof & De Grisse, 1989
Criconemoides incisus Raski & Golden, 1966
 = *Criconemella incisa* (Raski & Golden, 1966) Luc & Raski, 1961
 = *Macroposthonia incisa* (Raski & Golden, 1966) De Grisse, 1967
 = *Mesocriconema incisum* (Raski & Golden, 1956) Loof & De Grisse, 1989
Criconemoides informis (Micoletzky, 1922) Taylor, 1936
 = *Hoplolaimus informis* Micoletzky, 1922
 = *Criconemella informis* (Micoletzky, 1922) Ebsary, 1991
 = *Macroposthonia informis* (Micoletzky, 1922) De Grisse & Loof, 1965
Criconemoides kombaensis (Imamura, 1931) Taylor, 1936
 = *Criconema kombaense* Imamura, 1931

- = *Criconemella kombaensis* (Imamura, 1931) Ebsary, 1991
- = *Criconemella paragoodeyi* Choi & Geraert, 1975
- = *Criconemoides paragoodeyi* (Choi & Geraert, 1975) Loof & De Grisse, 1989
- Criconemoides obtusicaudatus* Heyns, 1962
- = *Mesocriconema obtusicaudatum* (Heyns, 1962) Loof & De Grisse, 1989
- Criconemoides onoensis* Luc, 1959
- = *Criconemella onoensis* (Luc, 1959) Luc & Raski, 1981
- = *Macroposthonia onoensis* (Luc, 1959) De Grisse & Loof, 1965
- = *Mesocriconema onoense* (Luc, 1959) Loof & De Grisse, 1989
- Criconemoides ornatus* Raski, 1958
- = *Criconemella ornata* (Raski, 1958) Luc & Raski, 1981
- = *Macroposthonia ornata* (Raski, 1958) De Grisse & Loof, 1965
- = *Mesocriconema ornatum* (Raski, 1958) Loof & De Grisse, 1989
- Criconemoides oryzae* (Sharma, Edward, Misra & Chandrashekar, 1992)
- = *Macroposthonia oryzae* Sharma, Edward, Misra & Chandrashekar, 1992
- = *Mesocriconema oryzae* (Sharma, Edward, Misra & Chandrashekar, 1992) Brzeski, Loof & Choi, 2002
- Criconemoides palustris* Luc, 1970
- = *Criconemella palustris* (Luc, 1970) Raski & Luc, 1981
- = *Mesocriconema palustre* (Luc, 1970) Loof & De Grisse, 1989
- Criconemoides paradenoudeni* (Rashid, Geraert & Sharma, 1987) Hunt, Luc & Manzanilla-López, 2005 in Luc, Sikora & Bridge, 2005
- = *Criconemella paradenoudeni* Rashid, Geraert & Sharma, 1987
- = *Macroposthonia paradenoudeni* (Rashid, Geraert & Sharma, 1987) Siddiqi, 2000
- = *Mesocriconema paradenoudeni* (Rashid, Geraert & Sharma, 1987) Loof & De Grisse, 1989
- Criconemoides paragoodeyi*
- cf. *Criconemoides kombaensis*
- Criconemoides parolineolatus* (Rashid, Geraert & Sharma, 1987) Hunt, Luc & Manzanilla-López, 2005 in Luc, Sikora & Bridge, 2005
- = *Criconemella parolineolata* Rashid, Geraert & Sharma, 1987
- = *Macroposthonia parolineolata* (Rashid, Geraert & Sharma, 1987) Siddiqi, 2000
- = *Mesocriconema parolineolatum* (Rashid, Geraert & Sharma, 1987) Ebsary, 1991
- Criconemoides pseudohercyniensis* De Grisse & Koen, 1964
- = *Criconemella pseudohercyniensis* (De Grisse & Koen, 1964) Raski & Luc, 1981
- = *Criconemoides morgensis* (Hofmänner in Hofmänner & Menzel, 1914) Taylor, 1936
- Criconemoides rusticus* (Micoletzky, 1915) Taylor, 1936
- = *Criconemella rustica* (Micoletzky, 1915) Luc & Raski, 1981
- = *Mesocriconema rusticum* (Micoletzky, 1915) Loof & De Grisse, 1989
- Criconemoides sphaerocephalus* Taylor, 1936
- = *Criconemella sphaerocephala* (Taylor, 1936) Luc & Raski, 1981
- = *Macroposthonia sphaerocephala* (Taylor, 1936) De Grisse & Loof, 1965
- = *Mesocriconema sphaerocephalum* (Taylor, 1936) Loof & De Grisse, 1989
- Criconemoides tescorum*
- cf. *Criconemoides curvatus* or *Mesocriconema curvatum*
- Criconemoides xenoplax* Raski, 1952
- = *Criconemella xenoplax* (Raski, 1952) Luc & Raski, 1981
- = *Macroposthonia xenoplax* (Raski, 1952) De Grisse & Loof, 1965
- = *Mesocriconema xenoplax* (Raski, 1952) Loof & De Grisse, 1989
- Crossonema*
- cf. *Ogma*
- Discocriconemella*** De Grisse & Loof, 1965 [Tyl., Criconematidae]
- Discocriconemella degrissei* Loof & Sharma, 1980
- Discocriconemella elettariae* Sharma & Edward, 1985
- Discocriconemella limitanea* (Luc, 1959) De Grisse & Loof, 1965
- Ditylenchus*** Filipjev, 1936 [Tyl., Anguinidae]
- Ditylenchus africanus* Wendt, Swart, Vrain & Webster, 1995
- Ditylenchus allii*
- cf. *Ditylenchus dipsaci*
- Ditylenchus angustus* (Butler, 1913) Filipjev, 1936
- Ditylenchus arachis* Zhang, Liu, Janssen, Zhang, Xiao, Li, Couvreur & Bert, 2014

- Ditylenchus destructor* Thorne, 1945
Ditylenchus dipsaci (Kühn, 1857) Filipjev, 1936
 = *Ditylenchus allii* (Beijerinck, 1883) Tarjan, 1960
 = *Ditylenchus fragariae* Kirjanova, 1951
Ditylenchus fragariae
 cf. *Ditylenchus dipsaci*
Ditylenchus gigas Vovlas, Troccoli, Palomares-Rius, De Luca, Liébanas, Landa, Subbotin & Castillo, 2011
Ditylenchus humuli Skarbilovich, 1872
Ditylenchus myceliophagus Goodey, 1958
Ditylenchus procerus (Bally & Reydon, 1931) Filipjev, 1936
Dolichodorus Cobb, 1914 [Tyl., Dolichodoridae]
Dolichodorus heterocephalus Cobb, 1914
Dolichodorus minor Loof & Sharma, 1975
- Ecuadorus** Siddiqi, 2002 [Tri.]
Ecuadorus westindicus (Rodríguez-M, Sher & Siddiqi, 1978) Siddiqi, 2002
 = *Allotrachodorus westindicus* (Rodríguez-M, Sher & Siddiqi, 1978) Rashid, De Waele & Coomans, 1986
 = *Nanidorus westindicus* Rodríguez-M, Sher & Siddiqi, 1978
Eutylenchus Cobb, 1913 [Tyl., Atylenchidae]
Eutylenchus africanus Sher, Corbett & Colbran, 1966
- Filenchus** Andrassy, 1954 [Tyl., Tylenchidae]
Filenchus heterocephalus Xie & Feng, 1996
- Globodera** Skarbilovich, 1959 [Tyl., Heteroderidae]
Globodera capensis Knoetze, Swart & Tiedt, 2013
Globodera ellingtonae Handoo, Carta, Skantar & Chitwood, 2012
Globodera leptonepia (Cobb & Taylor, 1953) Skarbilovich, 1959
Globodera pallida Stone, 1973
 = *Heterodera pallida* Stone, 1973
Globodera rostochiensis (Wollenweber, 1923) Skarbilovich, 1959
Globodera tabacum tabacum (Lownsbery & Lownsbery, 1954) Skarbilovich, 1959
 = *Globodera tabacum* (Lownsbery & Lownsbery, 1954) Skarbilovich, 1959
Globodera tabacum solanacearum (Miller & Gray, 1972) Behrens, 1975
 = *Globodera solanacearum* (Miller & Gray, 1972) Behrens, 1975
Globodera virginiae (Miller & Gray, 1968) Stone, 1973
 = *Globodera tabacum virginiae* (Miller & Gray, 1968) Stone, 1973
Globodera zelandica Wouts, 1984
Gracilacus Raski, 1962 [Tyl., Tylenchulidae]
Gracilacus peratica Raski, 1962
- Halenchus** Cobb in Cobb, 1933 [Tyl., Anguinidae]
Helicotylenchus Steiner, 1945 [Tyl., Hoplolaimidae]
 = *Rotylenchoides* Whitehead, 1958
Helicotylenchus abunaamai Siddiqi, 1972
Helicotylenchus affinis (Luc, 1960) Fortuner, 1984
 = *Rotylenchoides affinis* Luc, 1960
Helicotylenchus astriatus Khan & Nanjappa, 1972
Helicotylenchus brevis (Whitehead, 1958) Fortuner, 1984
 = *Rotylenchoides brevis* Whitehead, 1958
Helicotylenchus cavenessi Sher, 1966
Helicotylenchus coffae Eroshenko & Nguen Vu Thanh, 1981
Helicotylenchus crenacauda Sher, 1966
Helicotylenchus digitiformis Ivanova, 1967
Helicotylenchus digonicus Perry in Perry, Darling & Thorne, 1959
Helicotylenchus dihystra (Cobb, 1893) Sher, 1961
Helicotylenchus egyptiensis Tarjan, 1964
Helicotylenchus erythrinae (Zimmermann, 1904) Golden, 1956
Helicotylenchus indicus Siddiqi, 1963
Helicotylenchus intermedius (Luc, 1960) Siddiqi & Husain, 1964
 = *Rotylenchoides intermedius* Luc, 1960
Helicotylenchus microcephalus Sher, 1966
Helicotylenchus mucronatus Siddiqi, 1963
Helicotylenchus multicinctus (Cobb, 1893) Golden, 1956
Helicotylenchus neopaxilli Inserra, Vovlas & Golden, 1975
Helicotylenchus oleae Inserra, Vovlas & Golden, 1979
Helicotylenchus paracanal Sauer & Winoto, 1975
Helicotylenchus pseudorobustus (Steiner, 1914) Golden, 1956
Helicotylenchus seren Siddiqi, 1963
Helicotylenchus sharafati Mulk & Jairajpuri, 1975
Helicotylenchus variocaudatus (Luc, 1960) Fortuner, 1984
 = *Rotylenchoides variocaudatus* Luc, 1960

- Hemicriconemoides** Chitwood & Birchfield, 1957 [Tyl., Criconeematidae]
Hemicriconemoides californianus Pinochet & Raski, 1975
Hemicriconemoides chitwoodi Esser, 1960
Hemicriconemoides cocophillus (Loos, 1949) Chitwood & Birchfield, 1957
Hemicriconemoides gaddi (Loos, 1949) Chitwood & Birchfield, 1957
Hemicriconemoides kanayaensis Nakasono & Ichinohe, 1961
Hemicriconemoides mangiferae Siddiqi, 1961¹
Hemicriconemoides mehdii Suryawanshi, 1971
Hemicriconemoides snoeckii Van Doorselaere & Samsoen, 1982
Hemicriconemoides strictathecatus Esser 1961
Hemicycliophora de Man, 1921 [Tyl., Criconeematidae]
Hemicycliophora arenaria Raski, 1958
Hemicycliophora argiensis Khan & Nanjappa, 1972
Hemicycliophora attapadii Rahaman, Ahmad & Jairajpuri, 1996
Hemicycliophora chathamii Yeates, 1978
Hemicycliophora chilensis Brzeski, 1974
Hemicycliophora longicaudata Loos, 1948
Hemicycliophora loofi Maas, 1970
Hemicycliophora nudata
 cf. *Caloosia nudata*
Hemicycliophora parvana Tarjan, 1952
Hemicycliophora penetrans Thorne, 1955
Hemicycliophora poranga Monteiro & Lordello, 1978
Hemicycliophora similis Thorne, 1955
Hemicycliophora thienemanni (Schneider, 1925) Loos, 1948
Hemicycliophora typica de Man, 1921
Hemicycliophora utkali Ray & Das, 1981
Heterodera Schmidt, 1871 [Tyl., Heteroderidae]
Heterodera africana (Luc, Germani & Netscher, 1973) Mundo-Ocampo, Troccoli, Subbotin, Del Cid, Baldwin & Inserra, 2008
 = *Sarisodera africana* Luc, Germani & Netscher, 1973
 = *Afenestrata africana* (Luc, Germani & Netscher, 1973) Baldwin & Bell, 1985
Heterodera arenaria Cooper, 1955
Heterodera aucklandica Wouts & Sturhan, 1995
Heterodera australis Subbotin, Sturhan, Rumpenhorst & Moens, 2002
Heterodera avenae Wollenweber, 1924
Heterodera bifenestrata Cooper, 1956
Heterodera cajani Koshy, 1967
 = *Heterodera vigni* Edward & Misra, 1968
Heterodera carotae Jones, 1950
Heterodera ciceri Vovlas, Greco & Di Vito, 1985
Heterodera cruciferae Franklin, 1945
Heterodera cyperi Golden, Rau & Cobb, 1962
Heterodera delvii Jairajpuri, Khan, Setty & Govindu, 1979
Heterodera elachista Ohshima, 1974
Heterodera fici Kirjanova, 1954
Heterodera filipjevi (Madzhidov, 1981) Steiner & Stelter, 1984
Heterodera gambiensis Merny & Netscher, 1976
Heterodera glycines Ichinohe, 1952
Heterodera goettingiana Liebscher, 1892
Heterodera graminis Stynes, 1971
Heterodera hordecalis Anderson, 1975
Heterodera humuli Filipjev, 1934
Heterodera latipons Franklin, 1969
Heterodera lespedezae Golden & Cobb, 1963
Heterodera litoralis Wouts & Sturhan, 1996
Heterodera mani Mathews, 1971
Heterodera marioni
 cf. *Meloidogyne marioni*
Heterodera medicaginis Kirjanova in Kirjanova & Krall, 1971
Heterodera mediterranea Vovlas, Inserra & Stone, 1981
Heterodera mothii Khan & Husain, 1965
Heterodera oryzae Luc & Berdon Brizuela, 1961
Heterodera oryzicola Rao & Jayaprakash, 1978
Heterodera pakistanensis Maqbool & Shahina, 1986
Heterodera pallida
 cf. *Globodera pallida*
Heterodera pratensis Gäbler, Sturhan, Subbotin & Rumpenhorst, 2000
Heterodera punctata
 cf. *Punctodera punctata*
Heterodera raskii Basnet & Jayaprakash, 1984
Heterodera ripae Subbotin, Sturhan, Rumpenhorst & Moens, 2003
Heterodera sacchari Luc & Merny, 1963
Heterodera salixophila Kirjanova, 1969
Heterodera schachtii A. Schmidt, 1871
Heterodera skohensis Kaushal, Sharma & Singh, 2000
Heterodera sorghi Jain, Sethi, Swarup & Srivastava, 1982
Heterodera spinicauda Wouts, Schoemaker, Sturhan & Burrows, 1995
Heterodera sturhani Subbotin, 2015
Heterodera swarupi Sharma, Siddiqi, Rahaman & Ansari, 1999

- Heterodera trifolii* Goffart, 1932
Heterodera urticae Cooper, 1955
Heterodera ustinovii Kirjanova, 1969
Heterodera vigni
 cf. *Heterodera cajani*
Heterodera zae Koshy, Swarup & Sethi, 1971
Hirschmanniella Luc & Goodey, 1964 [Tyl., Pratylenchidae]
Hirschmanniella asteromucronata Rasjivin, Fernandez, Ortega & Quincosa, 1981
Hirschmanniella belli Sher, 1968
Hirschmanniella caudacrena
 cf. *Hirschmanniella mexicana*
Hirschmanniella diversa Sher, 1968
Hirschmanniella dubia
 cf. *Hirschmanniella magna*
Hirschmanniella furcata Razjivin, Fernandez, Ortega & Quincosa, 1981
Hirschmanniella gracilis (de Man, 1880) Luc & Goodey, 1964
Hirschmanniella imamuri Sher, 1968
Hirschmanniella indica
 cf. *Hirschmanniella mucronata*
Hirschmanniella kaverii
 cf. *Hirschmanniella mucronata*
Hirschmanniella magna Siddiqi, 1966
 = *Hirschmanniella dubia* Khan, 1972
Hirschmanniella mangalorensis
 cf. *Hirschmanniella mucronata*
Hirschmanniella marina Sher, 1968
Hirschmanniella mexicana (Chitwood, 1961) Sher, 1968
 = *Hirschmanniella caudacrena* Sher, 1968
Hirschmanniella microtyla Sher, 1968
Hirschmanniella miticausa Bridge, Mortimer & Jackson, 1984
Hirschmanniella mucronata (Das, 1960) Luc & Goodey, 1964
 = *Hirschmanniella indica* Ahmad, 1974
 = *Hirschmanniella kaverii* Sivakumar & Khan, 1982
 = *Hirschmanniella mangalorensis* Mathur & Prasad, 1971
Hirschmanniella nana
 cf. *Hirschmanniella oryzae*
Hirschmanniella nghetinhensis Eroshenko & Chau in Eroshenko, Tyau, Tkhan & Kan, 1985
Hirschmanniella obesa Razjivin, Fernandez, Ortega & Quincosa, 1981
Hirschmanniella ornata Eroshenko & Chau in Eroshenko, Tyau, Tkhan & Kan, 1985
Hirschmanniella oryzae (van Breda de Haan, 1902) Luc & Goodey, 1964
 = *Hirschmanniella nana* Siddiqi, 1966
Hirschmanniella shamimi Ahmad, 1972
Hirschmanniella spinicaudata (Schuurmans Stekhoven, 1944) Luc & Goodey, 1964
 = *Tylenchorhynchus spinicaudatus* Schuurmans Stekhoven, 1944
Hirschmanniella thornei Sher, 1968
Hirschmanniella truncata Razjivin, Fernandez, Ortega & Quincosa, 1981
Hoplolaimus von Daday, 1905 [Tyl., Hoplolaimidae]
 = *Basirolaimus* Shamsi, 1979
Hoplolaimus aegypti Shaflee & Koura, 1970
 = *Basirolaimus aegypti* (Shaflee & Koura, 1970) Shamsi, 1979
Hoplolaimus clarissimus Fortuner, 1973
 = *Basirolaimus clarissimus* (Fortuner, 1973) Shamsi, 1979
Hoplolaimus columbus Sher, 1963
Hoplolaimus dimorphicus Mulk & Jairajpuri, 1976
 = *Basirolaimus dimorphicus* (Mulk & Jairajpuri, 1976) Shamsi, 1979
Hoplolaimus dubius Chaturvedi & Khera, 1979
 = *Basirolaimus dubius* (Chaturvedi & Khera, 1979) Siddiqi, 1986
Hoplolaimus galeatus (Cobb, 1913) Filipjev & Schuurmans Stekhoven, 1941
Hoplolaimus indicus Sher, 1963
 = *Basirolaimus indicus* (Sher, 1963) Shamsi, 1979
Hoplolaimus magnistylus Robbins, 1982
Hoplolaimus pararobustus (Schuurmans Stekhoven & Teunissen, 1938) Sher, 1963
Hoplolaimus seinhorsti Luc, 1958
 = *Basirolaimus seinhorsti* (Luc, 1958) Shamsi, 1979
Hypsoperine
 cf. *Meloidogyne*
Ibipora
 cf. *Belonolaimus*
Longidoroides Khan, Chawla & Saha, 1978 [L.]
Longidorus (Micoletzky, 1922) Thorne & Swanger, 1936 [L.]
Longidorus africanus Merny, 1966
Longidorus apulus Lamberti & Bleve-Zacheo, 1977
Longidorus breviannulatus Norton & Hoffmann, 1975

- Longidorus elongatus* (de Man, 1876) Thorne & Swanger, 1936
Longidorus fursti Heyns, Coomans, Hutsebaut & Swart, 1987
Longidorus israelensis Peneva, Orion, Shlevin, Bar-Eyal & Brown, 1998
Longidorus laevicapitatus Williams, 1959
Longidorus leptcephalus Hooper, 1961
Longidorus maximus
 cf. *Paralongidorus maximus*
Longidorus pisi Edward, Misra & Singh, 1964
 = *Longidorus siddiqii* Aboul-Eid, 1970
Longidorus siddiqii
 cf. *Longidorus pisi*
Longidorus vineicola Sturhan & Weischer, 1964
- Macroposthonia**
 cf. *Criconemoides* or *Mesocriconema*
- Meloidodera** Chitwood, Hannon & Esser, 1956
Meloidodera alni
 cf. *Meloidodera sikhotealiniensis*
Meloidodera sikhotealiniensis Eroshenko, 1978
 = *Meloidodera alni* Turkina & Chizhov, 1986
- Meloidogyne** Goeldi, 1887 [Tyl., *Meloidogynidae*]
 = *Hypsoperine* Sledge & Golden, 1964
Meloidogyne acrita
 cf. *Meloidogyne incognita*
Meloidogyne acrona Coetzee, 1956
Meloidogyne africana Whitehead, 1960
 = *Meloidogyne decalineata* Whitehead, 1968
 = *Meloidogyne oteifai* Elmiligy, 1968 [emended spelling from original 'oteifae']
Meloidogyne arabicida López & Salazar, 1989
Meloidogyne arenaria (Neal, 1889) Chitwood, 1949
Meloidogyne arenaria thamesi
 cf. *Meloidogyne thamesi*
Meloidogyne artiellia Franklin, 1961
Meloidogyne baetica Castillo, Vovlas, Subbotin & Troccoli, 2003
Meloidogyne brasiliensis Charchar & Eisenback, 2002
Meloidogyne brevicauda Loos, 1953
Meloidogyne chitwoodi Golden, O'Bannon, Santo & Finley, 1980
Meloidogyne christiei Golden & Kaplan, 1986
Meloidogyne coffeicola Lordello & Zamith, 1960
Meloidogyne cruciani Garcia-Martinez, Taylor & Smart, 1982
Meloidogyne decalineata
 cf. *Meloidogyne africana*
Meloidogyne enterolobii Yang & Eisenback, 1983
 = *Meloidogyne mayaguensis* Rammah & Hirschmann, 1988
Meloidogyne ethiopica Whitehead, 1968
Meloidogyne exigua Goeldi, 1887
Meloidogyne fallax Karssen, 1996
Meloidogyne floridensis Handoo, Nyczepir, Es-menjaud, van der Beek, Castagnone-Sereno, Carta, Skantar & Higgins, 2004
Meloidogyne fujianensis Pan, 1985
Meloidogyne grahami
 cf. *Meloidogyne incognita*
Meloidogyne graminicola Golden & Birchfield, 1965
Meloidogyne graminis (Sledge & Golden, 1964) Whitehead, 1968
Meloidogyne hainanensis Liao & Feng, 1995
Meloidogyne hapla Chitwood, 1949
Meloidogyne haplanaria Eisenback, Bernard, Starr, Lee & Tomaszewski, 2003
Meloidogyne hispanica Hirschmann, 1986
Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949
 = *Meloidogyne incognita acrita* Chitwood, 1949
 = *Meloidogyne acrita* Chitwood, 1949
 = *Meloidogyne grahami* Golden & Slana, 1978
Meloidogyne inornata Lordello, 1956
Meloidogyne izalcoensis Carneiro, Almeida, Gomes & Hernández, 2005
Meloidogyne javanica (Treub, 1885) Chitwood, 1949
Meloidogyne kikuyensis De Grisse, 1961
Meloidogyne konaensis Eisenback, Bernard & Schmitt, 1995
Meloidogyne kongi Yang, Wang & Feng, 1988
Meloidogyne lini Yang, Hu & Zhu, 1988
Meloidogyne lopezi Humphreys-Pereira, Flores-Chaves, Gómez, Salazar, Gómez-Alpizar & Elling, 2014
Meloidogyne lusitanica Abrantes & Santos, 1991
Meloidogyne marioni (Cornu, 1879) Chitwood & Oteifa, 1952 (sp. inq.)
 = *Heterodera marioni* (Cornu, 1879) Marciniowski, 1909
Meloidogyne mayaguensis
Meloidogyne enterolobii
 cf. *Meloidogyne enterolobii*
Meloidogyne megadora Whitehead, 1958
Meloidogyne microcephala Cliff & Hirschmann, 1984
Meloidogyne microtyla Mulvey, Townshend & Porter, 1975

- Meloidogyne minor* Karssen, Bolk, van Aelst, van der Beld, Kox, Korthals, Molendijk, Zijlstra, van Hoof & Cook, 2004
- Meloidogyne moroccensis* Rammah & Hirschmann, 1990
- Meloidogyne naasi* Franklin, 1965
- Meloidogyne oryzae* Maas, Sanders & Dede, 1978
- Meloidogyne oteifai*
cf. *Meloidogyne africana*
- Meloidogyne ottersoni* (Thorne, 1969) Franklin, 1971
- Meloidogyne panyuensis* Liao, Tang, Feng & Karssen, 2005
- Meloidogyne paranaensis* Carneiro, Carneiro, Abrantes, Santos & Almeida, 1996
- Meloidogyne petuniae* Charchar, Eisenback & Hirschmann, 1999
- Meloidogyne piperi* Sahoo, Ganguly & Eapen, 2000
- Meloidogyne pisi* Charchar, Eisenback, Charchar & Boiteau, 2008
- Meloidogyne platani* Hirschmann, 1982
- Meloidogyne salasi* Lopez-Chaves, 1985
- Meloidogyne spartinae* (Rau & Fassuliotis, 1965) Whitehead, 1968
- Meloidogyne suginamiensis* Toida & Yaegashi, 1984
- Meloidogyne thailandica* Handoo, Skantar, Carta & Erbe, 2005
- Meloidogyne thamesi* Chitwood in Chitwood, Specht & Havis, 1952
= *Meloidogyne arenaria thamesi* Chitwood in Chitwood, Specht & Havis, 1952
- Meloidogyne triticoryzae* Gaur, Saha & Khan, 1993
- Merlinius** Siddiqi, 1970 [Tyl., Belonolaimidae]
- Merlinius brevidens* (Allen, 1955) Siddiqi, 1970
- Merlinius cylindricus* (Ivanova, 1962) Siddiqi, 1970
- Merlinius gatevi* Budurova, 1988
- Mesocriconema** Andrassy, 1965 [Tyl., Criconematidae]
- Mesocriconema axeste* (Fassuliotis & Williamson, 1959) Loof & De Grisse, 1989
= *Criconemoides axestis* Fassuliotis & Williamson, 1959
= *Criconemella axestis* (Fassuliotis & Williamson, 1959) Luc & Raski, 1981
- Mesocriconema brevistylus* (Singh & Khera, 1976) Loof & De Grisse, 1989
= *Criconemoides brevistylus* Singh & Khera, 1976
- Mesocriconema curvatum* (Raski, 1952) Loof & De Grisse, 1989
= *Criconemoides curvatus* Raski, 1952
= *Criconemella curvata* (Raski, 1952) Luc & Raski, 1981
= *Criconemoides tescorum* de Guiran, 1963
= *Macroposthonia tescora* (de Guiran, 1963) De Grisse & Loof, 1965
= *Mesocriconema tescorum* (de Guiran, 1963) Loof & De Grisse, 1989
- Mesocriconema denoudeni* (Heyns, 1962) Loof & De Grisse, 1989
= *Criconemoides denoudeni* Heyns, 1962
- Mesocriconema dherdei* (De Grisse, 1967) Loof & De Grisse, 1989
= *Macroposthonia dherdei* De Grisse, 1967
= *Criconemoides dherdei* (De Grisse, 1967) Luc, 1970
- Mesocriconema ferniae* (Luc, 1959) Loof & De Grisse, 1989
= *Criconemoides ferniae* Luc, 1959
= *Criconemella ferniae* (Luc, 1959) Raski & Luc, 1981
- Mesocriconema incisum* (Raski & Golden, 1956) Loof & De Grisse, 1989
= *Criconemoides incisus* Raski & Golden, 1966
= *Criconemella incisa* (Raski & Golden, 1966) Luc & Raski, 1961
= *Macroposthonia incisa* (Raski & Golden, 1966) De Grisse, 1967
- Mesocriconema obtusicaudatum* (Heyns, 1962) Loof & De Grisse, 1989
= *Criconemoides obtusicaudatus* Heyns, 1962
- Mesocriconema onoense* (Luc, 1959) Loof & De Grisse, 1989
= *Criconemoides onoensis* Luc, 1959
= *Macroposthonia onoensis* (Luc, 1959) De Grisse & Loof, 1965
= *Criconemella onoensis* (Luc, 1959) Luc & Raski, 1981
- Mesocriconema ornatum* (Raski, 1958) Loof & De Grisse, 1989
= *Criconemoides ornatus* Raski, 1958
= *Macroposthonia ornata* (Raski, 1958) De Grisse & Loof, 1965
= *Criconemella ornata* (Raski, 1958) Luc & Raski, 1981
- Mesocriconema oryzae* (Sharma, Edward, Misra & Chandrashekar, 1992) Brzeski, Loof & Choi, 2002
= *Macroposthonia oryzae* Sharma, Edward, Misra & Chandrashekar, 1992

= *Criconemoides oryzae* (Sharma, Edward, Misra & Chandrashekar, 1992)

Mesocriconema palustre (Luc, 1970) Loof & De Grisse, 1989

= *Criconemoides palustris* Luc, 1970

= *Criconemella palustris* (Luc, 1970) Raski & Luc, 1981

Mesocriconema paradenouden (Rashid, Geraert & Sharma, 1987) Loof & De Grisse, 1989

= *Criconemella paradenouden* Rashid, Geraert & Sharma, 1987

= *Macroposthonia paradenouden* (Rashid, Geraert & Sharma, 1987) Siddiqi, 2000

= *Criconemoides paradenouden* (Rashid, Geraert & Sharma, 1987) Hunt, Luc & Manzanilla-López, 2005 in Luc, Sikora & Bridge, 2005

Mesocriconema paralineolatum (Rashid, Geraert & Sharma, 1987) Ebsary, 1991

= *Criconemella paralineolata* Rashid, Geraert & Sharma, 1987

= *Macroposthonia paralineolata* (Rashid, Geraert & Sharma, 1987) Siddiqi, 2000

= *Criconemoides paralineolatus* (Rashid, Geraert & Sharma, 1987) Hunt, Luc & Manzanilla-López, 2005 in Luc, Sikora & Bridge, 2005

Mesocriconema rusticum (Micoletzky, 1915) Loof & De Grisse, 1989

= *Criconemoides rusticus* (Micoletzky, 1915) Taylor, 1936

= *Criconemella rustica* (Micoletzky, 1915) Luc & Raski, 1981

Mesocriconema sphaerocephalum (Taylor, 1936) Loof & De Grisse, 1989

= *Criconemoides sphaerocephalus* Taylor, 1936

= *Macroposthonia sphaerocephala* (Taylor, 1936) De Grisse & Loof, 1965

= *Criconemella sphaerocephala* (Taylor, 1936) Luc & Raski, 1981

Mesocriconema tescorum

cf. *M. curvatum*

Mesocriconema xenoplax (Raski, 1952) Loof & De Grisse, 1989

= *Criconemoides xenoplax* Raski, 1952

= *Macroposthonia xenoplax* (Raski, 1952) De Grisse & Loof, 1965

= *Criconemella xenoplax* (Raski, 1952) Luc & Raski, 1981

Monotrichodor

cf. *Trichodorus*

Monotrichodor *monohystera*

cf. *Trichodorus monohystera*

Nacobb Thorne & Allen, 1944 [Tyl., Pratylenchidae]

Nacobb *aberrans* (Thorne, 1935) Thorne & Allen, 1944

Nacobb *bolivianus* Lordello, Zamith & Boock, 1961

Nacobb *dorsalis* Thorne & Allen, 1944

Nanidor Siddiqi, 1974 [Tri.]

Nanidor *minor* (Colbran, 1956) Siddiqi, 1974

= *Trichodorus minor* Colbran, 1956

= *Paratrichodorus minor* (Colbran, 1956) Siddiqi, 1974

= *Paratrichodorus christiei* (Allen, 1957) Siddiqi, 1974

= *Trichodorus christiei* Allen, 1957

Neodolichodor Andrassy, 1976 [Tyl. Dolichodoridae]

Ogma Southern, 1914 [Tyl., Criconematidae]

= *Crossonema* Khan, Chawla & Saha, 1976

Ogma *decalineatum* (Chitwood, 1957) Andrassy, 1979

Ogma *rhombosquamatum* (Mehta & Raski, 1971) Andrassy, 1979

Ogma *taylatum* (Khan, Chawla & Saha, 1976) Siddiqi, 1986

= *Crossonema taylatum* Khan, Chawla & Saha, 1976

Panagrolaim Fuchs, 1930 [P.]

Panagrolaim *rigidus* (Schneider, 1866) Thorne, 1937

Paralongidor Siddiqi, Hooper & Khan, 1963 [L.]

= *Siddiqia* Khan, Chawla & Saha, 1968

Paralongidor *australis* Stirling & McCulloch, 1985

Paralongidor *citri* (Siddiqi, 1959) Siddiqi, Hooper & Khan, 1963

Paralongidor *lutensis* Hunt & Rahman, 1991

Paralongidor *maximus* (Bütschli, 1874) Siddiqi, 1964

= *Longidor* *maximus* (Bütschli, 1874)

Thorne & Swanger, 1936

Paralongidor *natalensis* (Jacobs & Heyns, 1982) Luc & Doucet, 1984

Paralongidor *oryzae* Verma, 1973

Paralongidor *zenobiae* Hunt & Rahman, 1991

Paratrichodor Siddiqi, 1974 [Tri.]

Paratrichodor *allius* (Jensen, 1963) Siddiqi, 1974

= *Trichodorus allius* Jensen, 1963

- Paratrichodorus anemones* (Loof, 1965) Siddiqi, 1974
 = *Trichodorus anemones* Loof, 1965
- Paratrichodorus christiei*
 cf. *Nanidorus minor*
- Paratrichodorus lobatus* (Colbran, 1965) Siddiqi, 1974
 = *Trichodorus lobatus* Colbran, 1965
- Paratrichodorus minor*
 cf. *Nanidorus minor*
- Paratrichodorus mirzai* (Siddiqi, 1960) Siddiqi, 1974
 = *Trichodorus mirzai* Siddiqi, 1960
- Paratrichodorus pachydermus* (Seinhorst, 1954) Siddiqi, 1974
 = *Trichodorus pachydermus* Seinhorst, 1954
- Paratrichodorus porosus* (Allen, 1957) Siddiqi, 1974
 = *Trichodorus porosus* Allen, 1957
- Paratrichodorus teres* (Hooper, 1962) Siddiqi, 1974
 = *Trichodorus teres* Hooper, 1962
- Paratrophurus** Arias, 1970 [Tyl., Belonolaimidae]
- Paratrophurus acristylus* Siddiqi & Siddiqui, 1983
- Pratylenchus** Micoletzky, 1922 [Tyl., Tylenchulidae]
- Pratylenchus besoeikianus* Bally & Reydon, 1931
- Pratylenchus curvatus* van der Linde, 1938 (*sp. inq.*)
- Pratylenchus hamatus* Thorne & Allen, 1950
- Pratylenchus lepidus* Raski, 1975
- Pratylenchus minutus* Linford in Linford, Oliveira & Ishii, 1949
- Pratylenchus projectus* Jenkins, 1956
- Peltamigratus**
 cf. *Aorolaimus*
- Pratylenchoides** Winslow, 1958 [Tyl., Pratylenchidae]
- Pratylenchoides leiocauda* Sher, 1970
- Pratylenchus** Filipjev, 1936 [Tyl., Pratylenchidae]
- Pratylenchus agilis*
 cf. *Pratylenchus scribneri*
- Pratylenchus alleni* Ferris, 1981
- Pratylenchus andinus* Lordello, Zamith & Boock, 1961
- Pratylenchus barkati* Das & Sultana, 1979
- Pratylenchus brachyurus* (Godfrey, 1929) Filipjev & Schuurmans Stekhoven, 1941
- Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941
- Pratylenchus crenatus* Loof, 1960
- Pratylenchus dasi* Fortuner, 1985
- Pratylenchus delattrei* Luc, 1958
- Pratylenchus exilis* Das & Sultana, 1979
- Pratylenchus fallax* Seinhorst, 1968
- Pratylenchus flakkensis* Seinhorst, 1968
- Pratylenchus goodeyi* Sher & Allen, 1953
- Pratylenchus gutierrezii*
 cf. *Pratylenchus panamaensis*
- Pratylenchus hexincisus* Taylor & Jenkins, 1957
- Pratylenchus indicus* Das, 1960 (*sp. inq.*)
- Pratylenchus jaehni* Inserra, Duncan, Troccoli, Dunn, Maia dos Santos, Kaplan & Vovlas, 2001
- Pratylenchus kumamotoensis* Mizukubo, Sugimura & Uesugi, 2007
- Pratylenchus loosi* Loof, 1960
- Pratylenchus mediterraneus* Corbett, 1983
- Pratylenchus minyus*
 cf. *Pratylenchus neglectus*
- Pratylenchus neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941
 = *Pratylenchus minyus* Sher & Allen, 1953
- Pratylenchus panamaensis* Siddiqi, Dabur & Bajaj, 1991
 = *Pratylenchus gutierrezii* Golden, López Ch. & Vilchez R., 1992
- Pratylenchus parazeae* Wang, Zhuo, Ye & Liao, 2015
- Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941
- Pratylenchus pinguicaudatus* Corbett, 1969
- Pratylenchus pratensis* (de Man, 1880) Filipjev, 1936
- Pratylenchus pseudocoffeae* Mizukubo, 1992
- Pratylenchus pseudopratenensis* Seinhorst, 1968
 = *Pratylenchus sefaensis* Fortuner, 1974
- Pratylenchus sativus* Kaul, 1985
- Pratylenchus scribneri* Steiner, 1943
 = *Pratylenchus agilis* (Thorne & Malek, 1968) Hernández, Jordana, Goldaracena & Pinochet, 2000
- Pratylenchus sefaensis*
 cf. *Pratylenchus pseudopratenensis*
- Pratylenchus singhi* Das & Sultana, 1979
- Pratylenchus speijeri* De Luca, Troccoli, Duncan, Subbotin, Waeyenberge, Coyne, Brentu & Inserra, 2012
- Pratylenchus sudanensis* Loof & Yassin, 1971
- Pratylenchus teres* Khan & Singh, 1974
- Pratylenchus thornei* Sher & Allen, 1953
- Pratylenchus vulnus* Allen & Jensen, 1951
- Pratylenchus zaeae* Graham, 1951

Punctodera Mulvey & Stone, 1976 [Tyl., Heteroderidae]

Punctodera chalcensis Stone, Sosa-Moss & Mulvey, 1976

Punctodera punctata (Thorne, 1928) Mulvey & Stone, 1976

= *Heterodera punctata* Thorne, 1928

Quinisulcius Siddiqi, 1971 [Tyl., Belonolaimidae]

Quinisulcius acutus (Allen, 1955) Siddiqi, 1971

= *Tylenchorhynchus acutus* Allen, 1955

Radopholus Thorne, 1949 [Tyl., Pratylenchidae]

Radopholus arabocoffeae Trinh, Waeyenberge, Nguyen & Moens, 2004

Radopholus bridgei Siddiqi & Hahn, 1995

Radopholus citri Machon & Bridge, 1996

Radopholus citrophilus

cf. *Radopholus similis*

Radopholus daklakensis Trinh, Waeyenberge, Nguyen & Moens, 2012

Radopholus duriophilus Nguyen, Subbotin, Madani, Trinh & Moens, 2003

Radopholus inaequalis Sauer, 1958

Radopholus musicola Stanton, Mundo-Ocampo, Baldwin & Kaplan, 2001

Radopholus rotundisemenus Sher, 1968

Radopholus similis Cobb, 1913

= *Radopholus similis similis* Cobb, 1913

= *Radopholus similis citrophilus* Huettel,

Dickson & Kaplan, 1984

= *Radopholus citrophilus* Huettel, Dickson &

Kaplan, 1984

Radopholus citrophilus

cf. *Radopholus similis*

Radopholus vangundyi Sher, 1968

Radopholus williamsi

cf. *Achlysiella williamsi*

Rhadinaphelenchus

cf. *Bursaphelenchus*

Rotylenchoides

cf. *Helicotylenchus*

Rotylenchulus Linford & Oliveira, 1940 [Tyl., Hoplolaimidae]

Rotylenchulus borealis Loof & Oostenbrink, 1962

Rotylenchulus macrorodatus Dasgupta, Raski & Sher, 1968

Rotylenchulus macrosoma Dasgupta, Raski & Sher, 1968

Rotylenchulus parvus (Williams, 1960) Sher, 1961

Rotylenchulus reniformis Linford & Oliveira, 1940

Rotylenchus Filipjev, 1936 [Tyl., Hoplolaimidae]

Rotylenchus buxophilus Golden, 1956

Rotylenchus caudaphasmodius Sher, 1965

Rotylenchus microstriatus Siddiqi & Corbett, 1983

Scutellonema Andrassy, 1958 [Tyl., Hoplolaimidae]

Scutellonema aberrans

cf. *Scutellonema clathricaudatum*

Scutellonema africanum Smit, 1971

*Scutellonema brachyurus*² (Steiner, 1938) Andrassy, 1958

Scutellonema bradys (Steiner & LeHew, 1933) Andrassy, 1958

= *Scutellonema blaberum* (Steiner, 1937)

Andrassy, 1958

Scutellonema cavenessi Sher, 1964

Scutellonema clathricaudatum Whitehead, 1960

= *Scutellonema aberrans* (Whitehead, 1960)

Sher 1961

Scutellonema magniphasma Sher, 1965

Scutellonema paralabiatum Siddiqi & Sharma, 1994

Scutellonema siamense Timm, 1965

Scutellonema unum Sher, 1964

Senegalonema Germani, Luc & Baldwin, 1984 [Tyl., Hoplolaimidae]

Siddiqia

cf. *Paralongidorus*

Subanguina Paramonov, 1967 [Tyl., Anguinidae]

= *Afrina Brzeski*, 1981

Subanguina wevelli (Van den Berg, 1985) Ebsary, 1991

= *Afrina wevelli* Van den Berg, 1985

Thecavermiculatus

cf. *Atalodera*

Thecavermiculatus andinus

cf. *Atalodera andina*

Trichodorus Cobb, 1913 [Tri.]

= *Monotrichodorus* Andrassy, 1976

Trichodorus borneoensis Hooper, 1962

Trichodorus christiei

cf. *Namidorus minor*

Trichodorus monohystera Allen, 1957

= *Monotrichodorus monohystera* (Allen, 1957) Andrassy, 1976

Trichodorus porosus

cf. *Paratrachodorus porosus*

- Trichodorus primitivus* (de Man, 1880) Micoletzky, 1922
- Trichodorus similis* Seinhorst, 1963
- Trichodorus viruliferus* Hooper, 1963
- Trophotylenchulus** Raski, 1957 [Tyl., Tylenchulidae]
- Trophotylenchulus obscurus* (Colbran, 1961) Cohn & Kaplan, 1983
- Trophotylenchulus piperis* Mohandas, Ravana & Raski, 1985
- Trophotylenchulus saltensis* Hashim, 1984
- Trophurus** Loof, 1956 [Tyl., Belonolaimidae]
- Trophurus imperialis* Loof, 1956
- Tylencholaimus** de Man, 1876 [T.]
- Tylencholaimus asymmetricus* Khan & Ahmad, 1994
- Tylenchorhynchus** Cobb, 1913 [Tyl., Belonolaimidae]
- Tylenchorhynchus acutus* Allen, 1955
- Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971
- = *Tylenchorhynchus martini* Fielding, 1956
- Tylenchorhynchus cicerus* Kakar, Khan & Siddiqi, 1995
- Tylenchorhynchus brassicae* Siddiqi, 1961
- Tylenchorhynchus brevilineatus* Williams, 1960
- = *Tylenchorhynchus indicus* Siddiqi, 1961
- Tylenchorhynchus capitatus* Allen, 1955
- Tylenchorhynchus cicerus* Kakar, Khan & Siddiqi, 1995
- Tylenchorhynchus clarus* Allen, 1955
- Tylenchorhynchus clavicaudatus* Seinhorst, 1963
- Tylenchorhynchus claytoni* Steiner, 1937
- Tylenchorhynchus contractus* Loof, 1964
- Tylenchorhynchus crassicaudatus* Williams, 1960
- Tylenchorhynchus cylindricus* Cobb, 1913
- Tylenchorhynchus dubius* (Bütschli, 1873) Filipjev, 1936
- = *Bitylenchus dubius* (Bütschli, 1873) Filipjev, 1934
- Tylenchorhynchus elegans* Siddiqi, 1961
- Tylenchorhynchus ewingi* Hopper, 1959
- Tylenchorhynchus iarius* Saha, Gaur & Lal, 1998
- Tylenchorhynchus indicus*
- cf. *Tylenchorhynchus brevilineatus*
- Tylenchorhynchus karnalensis* Saha, Singh, Lal & Kaushal, 2002
- Tylenchorhynchus latus* Allen, 1955
- Tylenchorhynchus leviterminalis* Siddiqi, Mukherjee & Dasgupta, 1982
- Tylenchorhynchus martini*
- cf. *Tylenchorhynchus annulatus*
- Tylenchorhynchus mashhoodi* Siddiqi & Basir, 1959
- Tylenchorhynchus nudus* Allen, 1955
- Tylenchorhynchus obtusus* (Siddiqi, 1978) Fortuner & Luc, 1987
- Tylenchorhynchus oryzae* Kaul & Waliullah, 1995
- Tylenchorhynchus queirozi* Monteiro & Lordello, 1976
- Tylenchorhynchus spinicaudatus*
- cf. *Hirschmanniella spinicaudata*
- Tylenchorhynchus swarupi* Singh & Khera, 1978
- Tylenchorhynchus vulgaris* Upadhyay, Swarup & Sethi, 1972
- Tylenchorhynchus zambiensis* Venditti & Noel, 1995
- = *Bitylenchus zambiensis* (Venditti & Noel, 1995) Siddiqi, 2000
- Tylenchorhynchus zae* Sethi & Swarup, 1968
- Tylenchulus** Cobb, 1913 [Tyl., Tylenchulidae]
- Tylenchulus graminis* Inserra, Vovlas, O'Bannon & Esser, 1988
- Tylenchulus musicola* Tanha Maafi, Amani, Stanley, Inserra, Van den Berge & Subbotin, 2012
- Tylenchulus palustris* Inserra, Vovlas, O'Bannon & Esser, 1988
- Tylenchulus semipenetrans* Cobb, 1913
- Tylenchus** Bastian, 1865 [Tyl., Tylenchidae]
- Xiphidorus** Monteiro, 1976 [L.]
- Xiphidorus minor* Rashid, Coomans & Sharma, 1986
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Notes

¹ Often regarded as a junior synonym of *H. strictathecatus*.

² Often cited as *brachyurum* in the literature.

³ Often cited as *Xiphinema brevicollum*, an emendation from the original name and one that is not universally accepted.

Index

Note: Page numbers in **bold** type refer to **figures**
Page numbers in *italic* type refer to *tables*

- abamectin 372
- abiotic stress factors 8, 137
- abundance 65, 78
 - tomato–maize rotation 78, **78**
- acerola 492, **492**
- acibenzolar-S-methyl 704, 727
- Acremonium implicatum* 661
- Africa, coffee 7, 554
- African nightshade, harvesting **830**
- African oil palm 510, 523
- agriculture
 - changes 6, 14
 - practical 1–3
- agroecosystems 548
 - soil health 77–82
- agroforestry 538
- agronomic practices 667–668
- air quality 62
- aldicarb 430, 456
 - cotton 745
 - Meloidogyne arenaria* 415
 - peanut 425
 - Pratylenchus thornei* 303
- alfatoxins 416
- alley cropping 808
- altitude 5, 667
- Amaranthus viridis* 380
- amendments 79–80, 372, 385
 - chitin 804
 - organic 330, 490, 802–805, 806
- American joint vetch 418
- ammonium nitrate 177
- amplified fragment length polymorphism (AFLP) 106, 109–110, 237
- anaerobic soil disinfestation (ASD) 362, 700
- Anguina* 27, 32
 - tritici* 32, **33**, 180–182, **180**, **181**, **182**
- anhydrobiosis 26–27
- animal feed 164, 321
- antagonistic crops 366, 374, 375, 602, 809–810, 811
- Aorolaimus* 43
 - luci* **44**
- Aphasmatylenchus* 45–46
 - nigeriensis* 46
 - straturatus* 5, **45**, 46, 321, 432
- Aphelenchoidae, morphology 20
- Aphelenchoides* 28–29
 - arachidis* 428–430
 - besseyi* 28–29, **28**, 126–129
 - rice 125–129, **126**
 - yam 271
 - ense* 622, 630
 - ritzemabosi* 316, 697
- arbuscular mycorrhizal fungi 264, 374, **823**, **823**
- Arecaceae 504
- arecanut 525–527, 528
- arecanut palm 514, **514**
- Arracacha 278
- Artemisia* 369, **371**
- ashwagandha 775–776, **776**
- at-planting management 821–823

- aubergine 227, 367, 373, 387
 fenamiphos 371
Globodera 379–380
Meloidogyne javanica 358
Solanum torvum 368
- avermectins 371
- avocado 477–478
- awl nematodes 387
- Bacillus* 176
firmus 373
subtilis 606, 760
- bacteria 372
 banana 631
Radopholus similis 760
Rhizobium 291
 sacrophytic 177
 sugarcane 678
- bacterial wilt 692, 696
- Baermann funnel technique 89–90, **91**
- Bambara groundnut 411, 434
- banana 5, 8, 9, 11, 38, 527, 617–618, 728, 759, 760, 819, **820**
 bacteria 631
 Banana Breeding Scheme 637
 biological control 639–640
 Breeding Better Bananas 636, 644
 chemical control 638–639
 climatic factors 631
 crop rotation 633
 cultivars 633, 635, 637, 645
 cultivated clones 617, 618
 cultivation techniques 619–621
 damage 623, 626
 endophytic colonization 639–640, **640**
 environmental factors 630–632
 exports 621, 635, 644
 extraction 642
 Fair Trade 645
 fallowing 632–633
 fungi 631–632
Fusarium wilt 632
 genetic modification 645
Helicotylenchus
microcephalus 630
mucronatus 630
multicinctus 621, 627–628, 631, 637, 641, **642**, 644
Heterodera oryzae 630
Hoplolaimus pararobustus 630
 hot water treatment 634–635
 intercropping 633–634, **634**
 international trade 618
 macropropagation plants 619, **621**
 management 641
Meloidogyne 628–629, 644
incognita 629, **629**
paranaensis 539
 mulching 633–634, **634**
 nematicides 632, 633, 638–639, **638**, 645
 non-export 621
 organic 638, 645
 amendments 80, **81**, 633–634
 parasites 631–632
 pesticides 637
Pratylenchus 621, 625–627, 644
coffae 625, 637, 644
goodeyi 35, 625
 production 80, **81**
 productivity methods 641, 641
 propagation techniques 618–619
Purpureocillium lilacinum 640
Radopholus similis 518, 622–625, **624**, 631, 636–637, 644, 803, **803**, 819, **820**, **821**
 resistance 635–638
 root
 rot 629
 system 631
Rotylenchulus reniformis 629–630
Rudopholus similis 632, 641, **642**
 sampling 641–642
 smallholders 618, 619
 soil
 suppressiveness 80, **81**
 type 630–631
 subsistence crop 621, **622**
 suckers 618, **619**, 631, 634–635
 systemic nematicides 827
 taro 800, **800**
 tissue culture plants 618–619, **620**
 toppling 621, 622, **622**, 628, 630, 632
 visual assessments 642–643, **643**
Xanthomonas bacterial wilt (BXW) 632, 644
 yield losses 632, 644
- banana weevil 631
- Barcode of Life Data Systems (BOLD) 110
- barley 163–186, 179, 183, 184, 807
Anguina tritici 182
Heterodera 168, 171, 807
 yield losses 183
- basal index 74
- bean 290
 mung 306–307
Nacobbus aberrans 232, 233
 velvet 673, 810
 see also broad bean; haricot bean
- Belonolaimus* 198, 358
 hosts 385
longicaudatus 384, 427, 427–428, **427**
 citrus 461–462, **462**
 cotton 746, **747**
 peanut 426–428, **427**
 yield losses 385

- management 385
- nematicides 385
- vegetables 384–385
- betel vine 755, 759, 772–774
- bioassays 231, 377, 706
- biocontrol agents 360
- biodiversity 7, 83, 678, 796
 - soil 330
- bioenhancement 823, **823**
- biofumigation 229, 703–704, 810–811, 813–816, **817**
 - Meloidogyne* 361–362, 810
 - tobacco 705
- bioindicators 64–65
- biological control agents 330
- biological nematicides 372
- biology 20
- biomass 65, 68–69, 76, 79–80, 677
- BioNem, tomato 373
- bionomics 23–27
- biotic stress factors 8, 137
- black gram 327–328
- black nightshade 365
- black pepper 527, 755–764
 - control
 - biological 760, 763
 - chemical 760, 763
 - cultural 759, 762
 - cultivars 760
 - Meloidogyne* 761–763, **761**
 - methyl bromide 762
 - Radopholus similis* 756–761
 - Rotylenchulus reniformis* 762
 - slow decline 756, **758**, 759
 - slow wilt disease 756, 759
 - Trophylenchulus piperis* 763
- black pod disease 566, 569
- black root rot 694
- black shank 692, 694, 696, **696**, 702
- Black Sigatoka 635, 644
- blast 127
- botanicals 607, 762
- Botryodiplodia theobromae* 255
- Bradyrhizobium* 322
 - japonicum* 324
- brahmi 776–777, **776**
- Brassica* 229, 361, 604, 705
 - junceae* 229
- breadfruit 492
- breeding programmes 11, 346, 796
- broad bean 292, 295–297, 298–299, 331
 - crop rotation 299
 - Ditylenchus* 292, 295–297, **295**, **296**
 - Heterodera* 297–299, **297**, **298**
 - Pratylenchus* 299
 - Radopholus similis* 299
 - Rotylenchulus reniformis* 299
 - Tylenchorhynchidae 299
- bucket-sieving method 94
- bunchy-top virus 644
- bunt 181
- burrowing nematodes 383
- Bursaphelenchus* 23, 29–30, 93
 - cocophilus* 508–512, **508**, 512, 514, 523
 - coconut 505–514, **507**
 - coconut palm 29, **30**
 - oil palm 519–523, **520**, **521**
 - xylophilus* 29, 112
- cabbage 380
- cabbage cyst nematode 380
- Cacopaurus* 24
- Cactodera* 380
- cadusafos 193, 361, 548
- Caloosia nudata* 463
- cantaloupe 377
- capacity building 12–13
- captan 459
- carbamate 238, 239, 456
- carbofuran 270, 373, 378, 430, 605
 - date palm 525
 - Meloidogyne incognita* 371
 - pomegranate 493
 - Rotylenchulus reniformis* 311
 - sugarcane 676
- carbohydrates 252
- carbon 65, 76, 79, 80
 - disulfide 370
 - soil 69
- carbosulfan 561
- cardamom 764–766
- carrot
 - Meloidogyne incognita* 352, **353**
 - Pratylenchus penetrans* 383, **383**, 384
- cash-crops 796, 806
- cashew 484–485, 484
- cassava 252–257
 - cultivars 252, 255, 256, 257
 - Glomus mosseae* 256
 - Meloidogyne* 253–256, **254**,
 - Pratylenchus* 256–257
 - Purpureocillium lilacinum* 256
 - Scutellonema bradys* 257, **257**
 - Tithonia diversifolia* 256
- castor 810
 - bean 311
 - oil 493
- cauliflower 380
- celery 352, **353**
- centres of excellence 13
- centrifugal flotation techniques 87, 95–96
- Ceratocystis paradoxa* 524

- cereal 163–221, 429
Anguina tritici 180–182
 crop rotation 199
 cultivars 184, 199
Ditylenchus 184–186
 fallow 184
Heterodera avenae 191
Meloidogyne 183, 199
Pratylenchus 176–180
 production 164, 164
 yield 164
 losses 199
- cereal cyst nematodes 164–176
see also Heterodera
- cereal–legume rotation 302
- challenges 1–19
- channel index 74, 79
- checkered leaf disease 697
- chemical fertilizers 74, 79
- chemical nematicides 10
- chickpea 299–304, 329
Heterodera 5, 302–303
Meloidogyne 299–302, **301**
Pratylenchus 303–304
Rotylenchulus 304
- Chinese water chestnut 278
- chitin amendments 360, 804
- chloride 165
- Chlorophytum borivillianum* 777
- chlorosis 330, 377, 380
 coconut 506
 coffee 545, 557
 cotton 739, 744
 soybean 322, 323, **323**, 327
 sugarcane 665
 tobacco 690, **691**
- Chromolaena*, yam 262
- citromela 452
- citrus 38, 446–476
Belonolaimus longicaudatus 461–462, **462**
 blight 446, 457
Caloosia nudata 463
 carbamate 456
 cultivars 451–452
Hemicycliphora arenaria 463
Meloidogyne 462–463
 nematicides 455–456
 organophosphate 456
Paratrichodorus 463
Pratylenchus 459, 460–461, **460**
Radopholus 457–460, **457**
 soil solarization 455–456
Trichodorus 463
Tylenchulus semipenetrans 447–456, **448**
Xiphinema 463
 yield losses 452, 453–454, 459
- citrus nematode *see Tylenchulus semipenetrans*
- Cladosporium* 124
- Clariva 176
- classification 20
 simplified outline 27
- clean fallow 802
- climate change 537, 545, 565, 589
- COAN 420
- cocoa 565–569
 cultivars 565–566, 567
Dolichodorus 568
Helicotylenchus 568
Meloidogyne 566–567, 569
Pratylenchus 568
 yield losses 566, 567, 569
- coconut 504–519, 528
Bursaphelenchus cocophilus 505–514, **507**
 chlorosis 506
 cultivars 506, 518
 germplasm 528
 mycorrhizae 517
Radopholus similis 514–518, 519
 red ring disease 505–508, **507**, 511, 512, 513–514
- coconut palm 504, 505
Bursaphelenchus cocophilus 29, **30**
- Coffea arabica* 536, 537, 553
Meloidogyne
aficana 555, **555**
exigua 540, **540**
incognita 540, 541, **541**, **542**
paranaensis 547, **547**
Pratylenchus panamaensis 557, **558**, 562, **562**
Radopholus arabicoffeae 563, **563**
 resistance 549–551, **551**, 561
- Coffea canephora* 536, 537
 genotypes 561–562
Meloidogyne incognita 540, **541**
Pratylenchus coffeae 540, **541**, 557, **559**
 resistance 548–549, **549**, **550**
- coffee 5, 7, 77, 536–565
 biological control 552
 chlorosis 545, 557
 climate change 537, 565
 corchosis 541, **543**
 crop rotation 551–552
 cropping systems 538
 cultivars 537, 539, 550
 cultivation techniques 538
 damage 554–555, 557, 559
 genome sequencing 564–565
 husk mulching 803, **805**
Impatiens 555
 integrated pest management 540
 intercropping 551–552
 leaf rust disease 537
Meloidogyne 538–556, **542**, 564
 nematicides 547–548
Pasteuria penetrans 552
Pratylenchus 556–562

- Radopholus similis* 563
- rootstock breeding programmes 549
- Rotylenchulus reniformis* 563–564
- seedlings 547, 564, 569
- tree coppicing 544
- Trophotylenchulus obscurus* 564
- yield losses 545–546, 555–556, 557
- Coleoptera 29
- collaboration 7
- Colocasia* 271–277, 276
- colonizer nematodes 64
- colonizer–persister groups 64, 64, 65, 69, 70, 71
- commercial farms 329
- common bean *see* haricot bean
- compost 67, 605
- computerized systems 101
- Consultative Group on International Agricultural Research (CGIAR) 176
- control 388, 795
- corchosis 541, **543**
- Corynebacterium* 260
 - michiganese* 180, **181**, 356
- cotton 179, 311, 315, 371, 738–747
 - Belonolaimus longicaudatus* 746, **747**
 - chemical control 743–744
 - chlorosis 739, 744
 - crop rotation 743, 745
 - cultivars 741, 743, 745
 - ectoparasitic nematodes 746
 - Fusarium* wilt 741–742, **742**
 - Hoplolaimus* 746
 - Longidorus* 747
 - Meloidogyne* 738–743, **739**, **740**, **741**
 - nematicides 742–743, 745
 - nematode population dynamics 740–741
 - peanut 418
 - Pratylenchus* 745–746
 - Rotylenchulus* 744–745, **744**, 801
 - seed treatment 742, 745
 - upland cotton 738
 - Xiphinema* 747
 - yield losses 743
- counting 67, 68
- cover crops 80–81, 290, 809, 810, **811**
 - cowpea 310
 - grape 490
 - kiwi 491
 - Meloidogyne* 365–366
 - pineapple 728–729
 - tea 601–602
- cowpea 46, 260, 307–312
 - Crotalaria spectabilis* 309
 - cultivars 309, 310, 311, 825
 - Heterodera* 310–311
 - Hoplolaimus seinhorsti* 312
 - Meloidogyne* 307–310, **308**, **309**
 - Pratylenchus brachyurus* 312
 - Rhizoctonia bataticola* 310
 - Rotylenchulus* 311–312
 - Trichoderma harzianum* 311
- Criconea* 148
- Criconeoides* 51–52, 148
 - curvatus* 512
 - pseudohercyniensis* **51**
- crop rotation 5, 10, 80–81, 163, 227, 329, 372, 374, 795, 805–806
 - banana 633
 - broad bean 299
 - cereal 199
 - chickpea 300, 301–302
 - coffee 551–552
 - cotton 743, 745
 - cowpea 309, 311
 - legume–cereal 302
 - maize–tomato 78, **78**
 - Meloidogyne* 137–138, 184
 - passionfruit 491, 492
 - pea 317
 - peanut 417–418, 425, 428, 430
 - pigeonpea 320
 - pineapple 728–729
 - potato 231, 234
 - Pratylenchus* 179, 275, 327
 - rice 124, 143–144, 147
 - Rotylenchulus reniformis* 326, 378
 - sugarcane 672–673, 679
 - sweet potato 237
 - tobacco 698–699, 707
 - yam 270
- cropping
 - alley 808
 - double 369
 - multiple 3, 808
 - patterns 796, **796**, 797, 800, 801
 - systems 10, 329, 357, 369, 372, 538
- crops
 - antagonistic 366, 374, 375, 602, 809–810, 811
 - cruciferous 361, 363, 380
 - fibre 658, 717, 738–754
 - graminaceous 323
 - management 362–369
 - non-food 687
 - non-host 363, 806–808, 807, 811
 - nut 484–485
 - perennial 546, 547, 565, 568–569, 599, 603, 800
 - production 13, 163, 228
 - residue management 69, 77–82
 - sequencing 328, 348
 - subtropical 4
 - transgenic 11
 - trap 228–229, 233, 365, 699–700, 808–809, 809, 811
 - tree 826, 827
 - tuber 8, 222, 252, 349, 352
 - vine 486–492, 827
 - see also* cover crops

- crop–livestock systems 222
- Crotalaria* 9, 309, 366, **811**
- cruciferous crops 361, 363, 380
- cryptic speciation 23, 35, 42
- cucumber 367, 369, 372, 373, 378
- cultivars 3, 5, 6, 7, 9, 10–12, 14, 163, 329, 346, 367, 388, 434, 813, 824, 825, 826, 827
- arecanut 526
- banana 633, 635, 637, 645
- cassava 252, 255, 256, 257
- chickpea 300, 303, 304
- citrus 451–452
- cocoa 565–566, 567
- coconut 506, 518
- coffee 537, 539, 550
- cotton 741, 743, 745
- cowpea 309, 310, 311, 825
- date 524
- fig 478–479
- haricot bean 314
- Meloidogyne* 367–368
- mixed 808
- olive 481, 482
- papaya 483, 484
- peanut 419, 420, 426, 428, 430–431, 434
- pepper 367, 760
- pigeonpea 320
- pineapple 719, 729–730
- potato 228
- soybean 321, 322–323, 324, 825
- sugarcane 658, 661, 674, 675, **675**
- sweet potato 237, 240
- taro 273
- tea 594, 595, 596, 603, 605, 607, 608
- tobacco 692, 694, 700–703, 705–706, 707
- tomato 367, 368–369, 825, **828**
- cultivation methods 10
- cultural practices 9
- Curvularia lunata* 127, 662–663
- Cylindrocladium black rot (CBR) 415, 416, 421
- cyst nematodes 68, 349, 379–387, 380
- vulval cones 100–101
- cyst-forming nematodes, diagnosis 330
- Dactylaria brochopaga* 138, 482
- date
- cultivars 524
- palm 504, 512, 523–525
- dazomet 361, 525
- dessication 26
- detection 87–119
- diagnostics 6–7, 14
- dibromochloropropane (DBCP) 518
- Discorea praeheensis* 260
- DiTera 727
- Ditylenchus* 31–32
- africanus*, peanut 431–432, **431**, 434
- angustus* 31, **31**, 122–123, 124
- rice 121–125, **122**, **123**, 149
- broad bean 292, 295–297
- cereal 184–186
- control 240
- destructor* 233–234, 381, 431
- sweet potato 240
- tobacco 697
- dipsaci* 5, 185, 185, 381–382, 820
- biology and life cycle 184–185, 381
- broad bean 292, 295
- garlic 381, 383
- hydrogen cyanide 382
- lentil 306
- management 185–186, 382–383
- oat 184, **184**, 185, 186
- oat race 381–382
- onion 381, 382
- pea 318
- potato 233, 234, **234**
- rye 185, 186
- sweet potato 240
- tobacco 697
- fumigation 297
- giant race 295, 296, 306
- gigas* 295, **295**, 296, **296**, 381
- oat race 295, 296, 297, 304
- potato 233–234
- sweet potato 240
- tobacco 697
- vegetables 380–383
- diversity
- genetic 62
- species 65, 67, 78
- DNA
- bar coding 110
- extraction 102
- fingerprinting 6
- sequencing 170
- Dolichodorus* 568
- heterocephalus* 278, 387
- double cropping 369
- drip-irrigation 10
- dry rot disease, yam 258–259, **259**, 260, 261, 265, 266
- Dryophthoridae 510
- durum wheat 171, 179
- ear cockle 181, 182
- ecology 62–86
- ecosystems
- functions 62, 76
- health 64
- services 77

- ectoparasites 25
 migratory 46, 52, 56
 ectoparasitic nematodes 746
 electricity 658, 677
 elephant grass 810
elicotylenchus, avocado 478
 elutriation techniques 95
 endoparasites 26, 34
 migratory 24, 35, 38, 43
 sedentary 24, 26
 endoparasitic nematodes 68
 endophytes 176
 endophytic fungi 375, 823
 enrichment index 74, 76, 79
 enset 618, 622, 626, 630
 environmental conditions 24
 enzyme-staining technique 170
 eradication programmes 227
Erianthus arundinaceus 674
 essential oils 705
 established crop management 824
 ethanol 196, 252, 677
 ethyl alcohol 658
 ethylene 718
Eupatorium, yam 262
 European and Mediterranean Plant Protection
 Organization (EPPO) 229
 exclusion 798
 extraction 87–119
 Bursaphelenchus 93
 from plant material 89–93
 from soil 93–96
 heteroderid cysts from dry soils 96
 materials 88
- Fair Trade 645
 fallowing 359, 802, 806
 banana 632–633
 cereal 184
 chickpea 300
 flood 418
 grape 488, 490
 pineapple 727–728
 sugarcane 672–673, 673, 679
 tobacco 699
- false root knot nematodes *see* *Nacobbus aberrans*
 farming
 cash-crop commercial 796
 commercial 329
 large-holder 3
 organic 608
 smallholder 1, 3, 5, 11, 566, 816
 subsistence 346, 357, 816
 systems 9, 10, 348, 796
- feeding-habit 69
 fenamiphos 361, 371, 456, 482, 493, 605, 704
- fertilizers 62, 74, 79–80, 829
 black pepper 759
 chemical 74, 79
 Meloidogyne arenaria 419
 nitrogen 79
 pineapple 723
 tea 601, 601, 607
 urea 195
- fibre crops 658, 717, 738–754
 field stratification 66
 fig 478–479
 filtration 87
 finger millet 199
 fixation, plant tissue and soil 88
 float beds 687, 703
 flood fallowing 418
 flooding 386, 801
 Meloidogyne 135, 136, 359
 rice 135, 136, 359
 tobacco 700
- flowers, cut 5
 fluensulfone 676, 704
 fluopyram 421, 676, 704
 fodder 196, 198, 252, 290, 305, 361
 Meloidogyne 366, 366
- foliar parasites, rice 120, 121–129
 food 1, 13, 658
 legumes 290, 291, 293, 294, 329, 330
 security 1, 3, 13, 222, 258, 796
 web system 65
- fossil fuels 62
 free-living nematodes 667, 668
 frosty pod disease 566
 fruit
 crops 477–484
 trees 492–493
- fuel 658
 fumigants 82, 358, 374, 604, 816
 Meloidogyne 370–371
 multispectrum 313, 318
- fumigation 482, 488, 703, 704, 723
 Pratylenchus 425, 703
 soil 348, 349, 800, 819
- functional guilds 64–65, 67, 69, 72, 76, 77
 funding 13
 fungi 8, 9, 127, 372
 arbuscular mycorrhizal 264, 374, 823, 823
 banana 631–632
 endophytic 375, 823
 mycorrhizal 455
 nematophagous 74, 80, 176, 184, 375
 pathogenic 671–672
 pineapple 730
 root-rotting 177
 sugarcane 678
 tea 591
 trapping 184

- fungicides 176, 459
 fungivorous nematodes 78, 668
 furfural aldehyde 676
Fusarium 124, 138, 198, 199, 239, 322, 425
 oxysporum 240, 300, 317, 640
 Meloidogyne incognita 318, 355–356, **356**, 741
 tomato 355–356, 375
Radopholus similis 758
sacchari 661
solani 416, 452, 479, 758–759
 wilt 318, 320, 321, 374, 632, 635, 692, 696, 698, 741–742, **742**, 825
- Galleria mellonella* 82
 garlic 381, 383
 genetics 10–12, 62, 730
 genome sequencing, coffee 564–565
 germplasm 171, 329, 564, 568
 giant swamp taro 277, **277**
 giant taros 277
 ginger 766–769
 global warming 544, 795
 globalization 386, 388, 795
Globodera 4, **48**, 49, 50
 aubergine 379–380
 biological control 229
 biology 224–226
 capensis 223
 damage 226–227
 diagnosis 229
 disease complexes 227
 economic importance 227–228
 ellingtonae 223
 environmental factors 227
 hosts 227
 management 228–229
 nematicides 229
 origin and distribution 223–224
 pallida 5, 223, **224**, 225, **225**, 226
 pathotypes 226
 Pochonia chlamydosporia 229, 231
 potato 223–229, 826
 rostochiensis 5, **48**, 223, **224**, 225, **225**
 species 223
 tabacum
 solanacearum 695–696, **695**, 705, 706
 virginiae 695
 tobacco 694–696
 tomato 379–380
 vegetables 379–387
 virulence 226
Glomus mosseae 256
 glucosinolates 229, 361
 gluten 196
 glycerine 98, 99
 glycerol method 99
Glycosmis pentaphylla 600
 good agricultural practices (GAPs) 607
 GPS-GIS technology 812
 grafting 368–369, 603, 828–829, **828**
 grain
 legumes 290
 sorghum 418
 see also barley; maize; wheat
 graminaceous crops 323
 granular nematicides 371, 817
 Granville wilt 692
 grape 451, 486–490, 489
 Meloidogyne 486, **486**, **487**, 488, 490
 Mesocriconema xenoplax 486–487, **487**
 Tylenchulus semipenetrans 486, 487, 488
 grape yellow vein virus 486
 grapefruit 452, 463
 grapevine fanleaf virus 486, 487, 488
 grasses 728
 grazing 365
 green cane trash blanket (GCTB) 678
 green manure 290, 292, 307, 367, 380, 527, **809**, **811**
 black pepper 759
 maize 195
 Meloidogyne 366, 366
 sugarcane 673
 tea 589
 groundnut 46, 240, 261, 365, 411–445, 673, 827
 Bambara 411, 434
 Mambara 434
 Meloidogyne arenaria 414, **414**
 Pratylenchus brachyurus 423, **423**
 guava 5, 479, **479**
- halogenated hydrocarbons 370
 handling 98
 haricot bean 312–316, **313**, **314**
 harvesting 830, **830**
 hatching 24–25
 heat treatments 820, 821
Helicotylenchus 25, 41–42, 485, 568, 599
 dihystera 667, 670
 microcephalus 630
 mucronatus 630
 multicinctus 42, **42**
 banana 621, 627–628, 631, 637, 641, **642**, 644
 biology and life cycle 628
 Fusarium oxysporum 640
 hosts 628
 plantain 627
 tannias 277
Hemicriconemoides 54, 479, 480, 598, 608
Hemicycliophora 25–26, 52–53, **53**, 54, 463

-
- henbanes 775
 herbicides 459, 594, 728
 herbivore pressure 76, 82
 herbs 755
 hermaphroditism 23
Heterodera 4, 6, 9, 48–49, 50, 164–176
 goettingiana
 broad bean 297, **297**, **298**
 Fusarium oxysporum 317
 hosts 298, 317
 management 298, 317
 mulching 317
 pea 316–317, **316**
 sacchari **48**, 144, 145, **145**, 146, 674
 zeae 191–192, 193, 195
 australis 165
 avenae 164, 802
 barley 168, 171
 cereal 191
 life cycle 165, **166**
 maize 191
 RAPD 106, **110**
 wheat 167, **167**, **168**, 171
 biological control 176
 biology and life cycle 165
 broad bean 297–299
 cajani 310, 311, 319, 319–320, **319**
 chickpea 302–303
 ciceri 5, 298, 302, 305, **305**
 cowpea 310–311
 cruciferae 380
 cultural practices 171
 damage 167–168, 170, 171
 distribution 164–165
 economic importance 146, 170–171
 elachista 144, 145, 146
 environmental factors 165
 fici 478
 filipjevi 165, 167, 168, 176
 glycines **48**, 324–325, **324**, 325
 cowpea 311
 haricot bean 314–315
 soybean 322–325, **324**, 825
 haricot bean 314–315
 hatching and survival 165, 167
 hordecalis 165
 hosts 145–146
 identification 170
 latipons 165, 167, 170, 171
 maize 191–192
 management 146, 171, 176
 mediterranea 482
 molecular diagnosis 146
 oryzae 9, **48**, 49, 144, 145
 oryzicola 144, 145, **145**, 146, 630
 pathotypes 168, 169, 170
 pea 316–317
 pigeonpea 319–320
 resistance 146, 171
 rice 144–146
 schachtii **48**, 311, 807, 812, **813**
 sturhani 165
 swarupi 303
 symptoms 145
 tobacco 697
 vegetables 380
 yield losses 170
 heteroderid cysts, extraction 96
 Heteroderidae 11
 Hirschmanniella 37–38, 139–144, 142, 149
 miticausa 5, 273–274, **274**
 oryzae **37**, 38, 140, **141**, 143, 144
 spinicaudata 143
 Hoplolaimus 43, **44**, 148, 312, 599, 630, 667, 741, 746
 hosts 24–26
 resistance 9, 10–11, 824–827
 hot water treatment 820
 banana 634–635
 Meloidogyne 270, 272
 palms 527–528
 Radopholus similis 527–528
 rice seed 128, 129
 Scutellonema bradys 263
 huanglongbing 446
 humidity 24
 Hunteria umbellata 485
 husbandry 829
 hydrogen cyanide, *Ditylenchus dipsaci* 382
 Hymenoptera 29
 hyperspectral sensors 812, **813**

 identification 23, 67, 68, 69, 98, 101, 110
 major genera 27–59
 molecular 82–83
 Impatiens 555
 indices
 basal 74
 channel 74, 79
 enrichment 74, **76**, 79
 maturity 68, 69, 72, 73, 74, 78
 plant parasitic 72
 soil food web 68, 74–76, 75
 structure 74, **76**, 78, 79
 infected tissue, removal 819–820
 information, dissemination 8
 insecticides 176
 integrated crop management (ICM) 795
 integrated nematode management (INM) 139, 329, 600
 strategies 830–831, **831**
 technologies 795–836
 integrated pest management (IPM) 517, 540, 600, 645, 795

- inter-cycle management 798, 800–801
intercropping 9, 163, 349, 538, 625, 796–797, **798, 800**, 900
 banana 633–634, **634**
 coffee 551–552
 cowpea 307, 309
 maize 194
 sugarcane 672–673, 679
 tea 599
 yam 261, 270
International Bureau of Plant Genetic Resources (IBPGR) 528
International Center for Agricultural Research in the Dry Areas (ICARDA) 302
International Cereal Nematodes Symposium (ICNS) 199
International Cereal Test Assortment 168, 169, 170, 171
International Maize and Wheat Improvement Center (CIMMYT) 199
Ipomoea 235
irrigation 415, 602
 drip- 10
isozyme methodologies 23
- jojoba oil, *Meloidogyne incognita* 490
jute 738, 749
- Kalahasti malady 430
kava 755, 774
kenaf 738, 747
kiwi 490–491, 491
knowledge transfer 14
- laboratories 7, 12, 13
lactoglycerol 98–99
lantana, cowpea 311
large-holder farms 3
legumes 5, 8, 46, 81, 144, 290, 673
 grain 290
 Meloidogyne artiellia 182–183
 Rhizobium bacteria 291
 yields 290–291, 292
 see also food, legumes
legume–cereal rotation 302
Leguminosae 310, 319
lentil 5, 298, 305–306, **305**
lesion nematodes
 diagnosis 331, 384
 see also *Pratylenchus*
lettuce 365, 387
life cycles 9, 68
life-history 69
lima bean 825
liquid nematicides 817
- little leaf disease 522
Longidoridae, morphology 20, **21**, 22–23
Longidorus 56, 57, **57**, 148–149, 198, 386–387, 747
loop-mediated isothermal amplification (LAMP) 112, **113**, 451, 528
loquat 492–493
lucerne 316
lupin 309, 425
lychee 479–480
- macadamia 485
maceration techniques 90–92
maize 8, 179, 182, 183, 186–196, 371, 425, 699
 botanicals 195–196
 control
 biological 196
 chemical 193
 cultural 194–195
 cowpea 309, 311
 crop rotation 194
 cultivars 193
 green manure 195
 Heterodera 191–192
 intercropping 194
 management 193–196
 Meloidogyne 186–189, **187, 188, 189**, 193, 195
 monocropping 195
 nematicides 193
 peanut 418
 PONCHO/VOTiVO 176
 Pratylenchus 189–191, **190**, 194, 195
 Punctodera chalconensis 192, 194
 resistance 193–194
 seed treatment 193
 sunflower compost 195
 Uganda 163
 Xiphinema 187, **189**
 yield losses 190–191, 192
maize–tomato rotation 78, **78**
Mallotus oppositifolius 485
Mambara groundnut 434
management 9–10, 163, 795–836
 at-planting 821–823
 cost-effective 795
 crop-based tools 805–811
 cropping based 362–369
 established crops 824
 physical methods 358–362, 801–805
 postharvest 829–830
 pre-plant 811–821
 tropics 3
mango 480
mangosteen 493
manure
 organic 705
 see also green manure

- mapping analysis 825
 marigold 309, 361, **362**, 366, 430, 728, 809, 810
 marine algae 24
 marker-assisted selection (MAS) 171, 194, 679, 825
 marotti oil cakes 527
 mash 327–328
 Mashua 278–279
 maturity
 index (MI) 64, 65, 78
 indices 68, 69, 72, 73, 74
 medicinal plants 755, 774–779, 809
Meloidogyne 7, 49–50, 182–184
 acrona 738, 739, 740, 741, **741**, 742
 africana 554, 555, **555**
 antagonistic crops 366
 arabica 554, 555, 556
 arbuscular mycorrhizal fungi 374
 arenaria **50**, 350, 555, 701
 acerola 492
 aldicarb 415
 cassava 253
 coffee 553
 cowpea 307
 fertilizers 419
 grape **487**
 groundnut 414, **414**
 haricot bean 312
 kiwi 490
 maize 188
 nematicides 416, 693
 olive 481
 Pasteuria penetrans 421–422, **422**
 peanut 412–418, **413**, **414**, 419, 420, 423
 pearl millet 198, 199
 Sclerotium rolfsii 415
 soybean 322
 tea 593
 tobacco 688, 690, 692, 693
 tomato 354, 359
 yam 268
 yield losses 416, 417
 artiellia 184, 301, 352
 chickpea 300–302, **301**
 legumes 182–183
 pea 317
 wheat 183
 avocado 478
Bacillus firmus 373
baetica 481
 banana 628–629, 644
 bioassays 231
 biofumigation 361–362
 biological control 270, 372–375, 421–422
 biological races 354, 355, 414–415
 biology and life cycle 130–132, 183, 187, 230,
 236–237, 268, 353–354, 629
brasiliensis 312, 317
brevicauda 594–596, 595, **595**, 608
 cardamom 764–766, **764**
 cassava 253–256
 cereal 199
 chemical control 270, 369–372, 547–548
 chickpea 299–302
chitwoodi 183, 230, **230**, 231, 312
 citrus 462–463
 cocoa 566–567
 coffee 538–556
coffeicola 547, 553, 554, 555
 conservation agriculture practices 136
 cotton 738–743
 cover crops 365–366
 cowpea 307–310
 crop rotation 363–365
 cultivars 367–368
 cultural control 269–270
 damage 183–184, 188–189, 230, 253–255,
 267–268, **267**, 352, 412–414,
 554–555, 594, 690
 date palm 523–524
 detection 608
 diagnosis 11, 139, 231, 238, 376–377, 422–423
 disease complexes 231, 255, 268, 355–356,
 415–416, 662–663, 692–693
 distribution 182–183, 186–187
 economic importance 133–135, 183–184,
 188–189, 231, 237, 268–269,
 356–357
 endophytic fungi 375
enterolobii 350, 555
 cassava 253
 coffee 553
 cotton 738
 guava 479
 haricot bean 312
 sweet potato 235, 238
 tomato 350, **350**
 environmental factors 230–231, 354, 415,
 594, 662
ethiopica 351, 662
 exclusion 547
exigua **50**, 543, 544, 545, 560–561
 cocoa 567
 Coffea arabica 540, **540**
 coffee 539–552, 564
 damage 540, 554, 555
 nematicides 547–548
 Pasteuria penetrans 552
 resistance 548–551
 rhizobacteria 544
 Rhizobacteria solani 545
 extraction 422–423
 fallow 359
 field sampling 377
 flooding 135, 136, 359

Meloidogyne (continued)

- floridensis* 350
- fodder 366, 366
- fumigant nematicides 370–371
- galling 352
- ginger 766–769
- graminicola* **50**, 130, 134, 137, 138, 800, 801
 - Bacillus megaterium* 138
 - crop rotation 137–138
 - onion 138
 - rice 129–130, **129**, **130**, **131**, 132, **132**, 133, **135**, 136, 138, 139, **140**, 149
 - wheat 136, 183
- graminis* 183
- grape 486, 488
- green manure 366, 366
- guava 479
- hapla* **50**, 350, 554–555, 688, 702
 - abamectin 372
 - cassava 253
 - coffee 553
 - cut flowers 5
 - ginger 767
 - haricot bean 312
 - kiwi 490, 491
 - peanut 412, 414, 416, 423
 - sweet potato 235
 - tea 593
 - temperature 353, 354, 415
 - tobacco 688, 690, 693
- haricot bean 312–314, **313**
- herbicides 594
- hosts 133, 230–231, 268, 354, 355, 567, 699, 762
- hot water treatment 270, 272
- incognita* **5**, **50**, 230, 236, 350, 357, 365, 543, 545
 - abamectin 372
 - acerola 492
 - banana 629, **629**
 - betel vine 772–774
 - biofumigation 810
 - biology and life cycle 541, 543
 - black pepper 761, 762
 - Botryodiplodia theobromae* 255
 - carbofuran 371
 - carrot 352, **353**
 - cassava 253, 254, **254**, 255
 - celery 352, **353**
 - chickpea 300
 - cocoa 566–567, 569
 - Coffea* 540, 541, **541**, **542**
 - coffee 539–552
 - cotton 738–739, **739**, **740**, 741, 743
 - cowpea 307, **308**, 309, **309**, 310
 - damage 540–541, 567
 - date palm 523, 524
 - fig 478
 - Fusarium oxysporum* 318, 355–356, **356**, 741
 - ginger 766–767, 768
 - Glycosmis pentaphylla* 600
 - grape 486, **486**, 488, 490
 - haricot bean 312, 313, 314, **314**
 - Hoplolaimus columbus* 741
 - jojoba oil 490
 - kiwi 490, 491
 - lima bean 825
 - maize 186, 188, 193, 195
 - mung bean 306
 - Myrothecium verrucaria* 704
 - olive 480, 481
 - Orobancha ramosa* 692
 - papaya 483–484, **483**
 - passionfruit 491
 - Pasteuria penetrans* **823**
 - pea 317
 - pearl millet 198, 199
 - pepper 352, **352**
 - pesticides 693
 - pineapple 729–730
 - pistachio 485
 - Pratylenchus* 661, 740–741
 - Purpureocillium lilacinum* 372–373
 - Pythium graminicola* 662, 663
 - resistance 310, 548–551, 568, 701
 - Rhizoctonia solani* 356
 - rice 135
 - Rotylenchulus reniformis* 741
 - soil solarization 238
 - soil suppressiveness 82
 - sorghum 197
 - soybean 322
 - sugarcane 662, 663, 665, **666**
 - sweet marjoram 490
 - sweet potato 236
 - taro 272, 273
 - tea 593, 594
 - temperature 821
 - tobacco 688, 690, 703, 826
 - tomato 130, 352, **352**, **353**, 354, 366, 368, 373, 375
 - turmeric 770–771
 - wheat 184
 - yam 268, 270
- infection and histopathology 412
- integrated management 270–271
- irrigation water 355
- izalcoensis* 554, 555
- javanica* **5**, **50**, 350, 555, 688, 701, 719–720
 - acerola 492, **492**
 - aubergine 358
 - cassava 253, 256

- Ceratocystis paradoxa* 524
 chickpea 300
 cocoa 567
 cowpea 307, **308**, 309, 310
Curvularia lunata 662–663
 date palm 523
 DBCP 256, 257
 ginger 767
 haricot bean 312, 313, 314
 lychee 480
 maize 187, 188, **189**, 193
 mustard 490
 nematocides 693
 olive 480, 481, **481**
 passionfruit 491, 492
Pasteuria penetrans 422
 peanut 412, 414, 415, 416, 417
 pearl millet 198, 199
 pineapple 718–721, **719**, **720**, 729
 pistachio 485
Pratylenchus zeae 667
 soil fumigation 256
 solarization 361
 soybean 322
 sugarcane 662, 663, 674
 taro 272, 273
 tea 593
 temperature 354, 415, 720
 tobacco 688, 690, 692, 693
 turmeric 770–771
 yam 270
 yield losses 416, 663
kikuyensis 662
konaensis 553
lopezi 553
 maize 186–189, **187**, **188**
 management 135–139, 184, 231, 237–238, 256, 269–271, 272–273, 375–376, 556
 marigold 361, **362**
mayaguensis 479
 mint 777–779
 molecular diagnosis 552
 morphologic and isoenzymatic diagnosis 552
 mung bean 306–307
naasi 182, 183, **183**
 nematocides 189, 369
 non-fumigant nematocides 371–372
offeicola 544
 organic amendments 360
oryzae 130, 132, 136
 papaya 482, 483
paranaensis 541, 543, 545, 548–551, 554
 banana 539
 Coffea arabica 547, **547**
 coffee 539–552, **542**, 553
 pathotypes 188
 pea 317–318
 peanut 412–423
Phytophthora nicotianae 692
 pigeonpea 320
 pineapple 9, 718–721
 pistachio 485
 planting date 359
Pochonia chlamydosporia 373
 population dynamics 133
 posterior cultural patterns 100
 potato 229–231
 publications 4, **4**
Purpureocillium lilacinum 372
 races 230
Ralstonia solanacearum 356
 resistance 137, 270, 367–368, 369, 419–421, 548–549
 rice 129–139, **131**
 root
 destruction 359
 galling rating scheme 376–377, **376**
 seedlings 352
 soil
 heating 360–361
 moisture 355, 415
 pH 354
 solarization 138
 tillage 359
 solarization 360–361, 363
 sorghum 197–198
 soybean 322–323
 sugarcane 662–663, **662**, 665, 668, 674, **675**
 survival and dissemination 132, 268, 354–355, 415, 690, 692, 741
 sweet potato 235–238, **236**
 symptoms 129–130, 187–188, 235–236
Tagetes 366
 tannias 276
 tea 593–596
 temperature 353–354
 tobacco 688–693, 689, **690**, **691**
 tomato 361, **362**
Trichoderma 373
tritricoryzae 130, 133, 136, 139
 tubers 352
 turmeric 770–771
 vegetables 349–377, 351
 weeds 369, 370
 winged bean 328
 yam 266–271, **267**, **269**
 yield losses 357
 melon 260
 menthol mint 777, 778–779, **778**, 779
Mesocriconema
 ornatum 432
 sphaerocephalum **51**
 xenoplax **51**, 486–487, **487**
 metabarcoding 23

- metabolic footprints 65, **66**, 67–68, 67, 69, 72, 74–76, 79, 82, **83**
 diversity-weighted 68
 soil food web 76, **77**
- metam sodium 371, 374, 455, 703
- methyl bromide 348, 388, 547, 703, 762
- methyl isothiocyanate 370
- microbivore nematodes 67
- microfaunal food web 78
- microhabitats 63
- migratory ectoparasites 46, 52, 56
- migratory endoparasites 24, 35, 38, 43
- millet 133, 198–199, 311, 320
 pearl 418
- mineralization 74, 76, 81, 82
- mint 777–779
- mistifier technique 90, **92**
- miti-miti 273, **274**
- mixed-cropping systems 797
- modified Baermann technique 93–94
- molecular diagnostic tools 7, 101–113, 796
- molecular identification 82–83
- molecular markers 23, 170, 825
- molecular techniques 23, 87, 170, 346, 548
- monilia pod rot 566, 569
- monocropping 195, 279, 348
- monoculture 349
- morphology 20–23
- mounting 99–101
- mtDNA genes 101–102
- mulching 69, 261, 360, 361, 371, 814, 816
 avocado 477
 banana 633–634, **634**
 coffee husk 803, **805**
Heterodera goettingiana 317
 papaya 483
 plantain 633–634, **635**
 plastic 816, **816**, **817**
 sesame straw 803, **804**
- multicropping 3, 228, 357, 759, 808
- multiple species infection 12
- multispectrum fumigants 313, 318
- mung bean 306–307
- mustard 311, 430, 490, 600, 705, 706, 728
- mycorrhizae, coconut 517
- mycorrhizal fungi 455
 arbuscular 264, 374, 823, **823**
- Myrothecium verrucaria* 704
- Nacobbus* 40–41, 358
aberrans **40**, 228, 232, 233, 379
 bean 232, 233
 haricot bean 315–316
 Mashua 279
Pochonia chlamydosporia 233
 potato 231–233, **232**
 sugarbeet 232, 233
 trap crops 233
 vegetables 379
dorsalis 41
- Nanidorus* **58**, 59
- neem 262, 311, 360, 361, 373, 378, 386, 600, 762, 810
Heterodera zeae 195
Radopholus similis 526, 527
- NemaTAM 420
- nematicides 3, 82, 349, 434, 816–819, 818
 arecanut 527
 banana 632, 633, 638–639, **638**, 645, 827
Belonolaimus 385, 428
 biological 372
 black pepper 760
 cardamom 765
 chemical 10
 citrus 455–456
 cocoa 568
 coconut 527
 coffee 547–548
 cotton 742–743, 745
 fig 478
 food legumes 330
 fumigant 370–371, 816
Globodera 229, 705, 706
 granular 371, 817
 grape 490
 haricot bean 314, 316
 liquid 816
 maize 193
 mango 480
Meloidogyne 189, 369, 416, 547–548, 693
 non-fumigant 371–372, 421, 704, 816, 817–818, 827
 olive 482
 papaya 483
 pea 318
 peanut 418, 421
 pineapple 726–727, 732
 pistachio 485
 pomegranate 493
 post-planting 827
Pratylenchus 147, 191, 426, 561
Radopholus similis 459, 639
 removal 795, 817
 rice 149
Rotylenchulus 239, 378
Rotylenchus 326
 seed treatment 371–372
 sugarcane 665, **666**, 670, 670, 673, 676, 677, 678, **678**, 679, 817
 sweet potato 238
 systemic 827
 tea 604, 605
 tobacco 703–704, 707

- Tylenchulus semipenetrans* 454
 vegetables 358, 388
 nematode community analyses 65–74
 Nematode Joint Indicator Analysis (NINJA) 67–68
 nematode management agents (NMAs) 707
 nematode wool 295
 nematodes, as bioindicators 64–65
 nematologists 6, 10, 12, 13
 nematology 1–3, 12, 13–14, 16, 17
 nematophagous fungi 74, 80, 176, 184, 375
 Nimitz 548
 nitrogen 66, 81, 165, 291, 806, 829
 fertilizers 79
 fixing 292, 324
 Pratylenchus thornei 177
 soil 144
 non-food crops 687
 non-fumigant nematicides 371–372, 421, 704, 816, 817–818, 827
 non-host crops 363, 806–808, 807, 811
 non-parasitic nematodes 177
 nut crops 484–485

 oat 164, 171, 182, 183, 184, **184**, 185, 186
 oca 278
 oil cakes 144, 600
 oil palm 519–523
 African 510, 523
 Bursaphelenchus cocophilus 519–523, **520**, **521**
 little leaf disease 522
 red ring disease 519–520, **520**, 521–523
 okra 356
 olive 451, 480–482
 Olluco 278
 onion 133, 138, 381, 382
 Ontario peach decline 26
 orchards 455, 459
 organic amendments 330, 490, 802–805, 806
 banana 633–634
 black pepper 761
 Meloidogyne 360
 sugarcane 673–674
 organic farming 608
 organic matter 74, 78, 80, 81, 705
 banana 80, **81**
 decomposition 79
 soil 667–668
 tea 600–601
 organophosphates 238, 239, 456
 ornamental palms 527, 528
 ornamentals 625
Orobancha ramosa 692
 oxamyl 264, 371, 373, 525, 676, 704, 745
 Pratylenchus coffeae 266
 Radopholus similis 459

Pachymetra 670, 671, 671
 paddy rice 304, 309, 311, 359, **360**, 801
 paddy rice–vegetable cropping systems 354
 palisade grass 240
 palm weevil 29, 504, 506, 508–512, **508**, **509**, 513, 521, 523, 524, 528
 palms 24, 504, 525, 526, 527–528
 Panama disease 632, 635
 papaya 482–484
Paralongidorus 56, 57, 148–149, 386–387
 parasites, banana 631–632
 parasitism 9, 24–25
Paratrichodorus 59, 235, 271, 385–386, 463, 668, 670
Paratylenchus curvatus 599
 partridge pea 418
 passionfruit 491–492
Pasteuria 176, 373–374
 nishizawae 176
 penetrans 372, 373–374, 421–422, **422**, 552, 605, **823**
 Radopholus similis 517
 Tylenchulus semipenetrans 456
 pasture 198, 290, 699
 pathogenic fungi 671–672
 pathogenicity 7–8
 pathotypes 26
 PCR 102–103, **102**, 135, 178, 238, 676
 real-time 111–112, **111**
 sequencing 103–105
 species-specific primers 105, **106**, 107, 108
 PCR-RFLP 103, **105**, 170, 239
 pea 296, 316–318, **316**
 straw 316
 peach 26
 peanut 411, 412–433
 aldicarb 425
 Aphasmatylenchus straturatus 432
 Aphelenchoides arachidis 428–430
 Belonolaimus longicaudatus 426–428, **427**
 cotton 418
 crop rotation 417–418, 425, 428
 cultivars 419, 420, 426, 428, 430–431, 434
 Ditylenchus africanus 431–432, **431**, 434
 fluopyram 421
 fumigants 421
 harvesting 422, 425
 maize 418
 Meloidogyne 412–423
 arenaria 412–418, **413**, **414**, 419, 420, 423
 hapla 412, 414, 416, 423
 javanica 412, 414, 415, 416, 417
 Mesocriconema ornatum 432
 nematicides 418, 421
 Pasteuria penetrans 421–422
 ploughing 418

- peanut (*continued*)
- pod
 - lesions 424
 - rot 416
 - Pratylenchus* 423–426
 - Scutellonema cavenessi* 432
 - shelling 430
 - tropical forages 417
 - Tylenchorhynchus brevilineatus* 430–431, 434
 - yield losses 414, 416, 417, 423, 424, 425, 428, 430
- pearl millet 198–199, 418
- Pedaliaceae 310, 319
- peg rot disease 423, 425
- penetration 24–25
- Penicillium* 425
- pepper 350, 359, 367, 414, 760, 823
 - Meloidogyne incognita* 352, **352**
 - yellow's disease 756, 758, 759
 - see also* black pepper
- perennial crops 546, 547, 565, 568–569, 599, 603, 800
- persimmon 484
- persister nematodes 64
- pest management 3, 9, 74, 76
- pesticides 10, 62, 74, 77, 81–82, 817, 823
 - banana 637
 - Meloidogyne incognita* 693
 - yam 264
- Phytophthora* 446, 505
- Phytophthora nicotianae* 452, 454, 455–456, 692
- phytotoxicity 455
- pigeonpea 46, 318–321, **319**
- pine 24, 29
- pineapple 717–737
 - cover crops 728–729
 - crop rotation 728–729
 - cultivars 719, 729–730
 - cultivation techniques 717–718
 - cultural practices 727
 - damage 719, 721, 723–724
 - fertilizers 723
 - fumigation 723
 - fungi 730
 - Meloidogyne* 9, 718–721, 729–730, **719, 720**
 - nematicides 726–727, 732
 - Pratylenchus* 723–726
 - Purpureocillium lilacinum* 730
 - read leaf disease 725
 - Rotylenchulus reniformis* 721–723, **722, 819**
 - yield losses 718, 719, 721, 723, 725, 731
- pineapple mealybug wilt 723
- pistachio 485
- plant growth-promoting rhizobacteria (PGPR) 704
- plant health 4, 62–63
- plant material
 - direct examination 88–89
 - extraction from 89–93
- plant parasitic index (PPI) 72
- plantain 8, 617–618, 819
 - Helicotylenchus multicinctus* 627
 - Hoplolaimus pararobustus* 630
 - mulching 633–634, **635**
 - Pratylenchus coffeae* 625, 627, 632
 - suckers 634, **635**
 - yield losses 632
 - see also* banana
- planting date 821, 822
- plastic mulch 816, **815, 817**
- ploughing 418, 698
- Pochonia chlamydosporia* 229, 231, 233, 360, 373, 485
- pollution 62
- polymerase chain reaction *see* PCR
- pomegranate 451, 493
- PONCHO/VOTIVO, maize 176
- Poorman orange 452
- post-harvest management 829–830
- post-planting nematicide treatment 827
- potash 601, **601**, 607
- potato 222–235
 - crop rotation 231, 234
 - Ditylenchus* 233–234, **234**
 - Globodera* 5, 223–229, **225**, 826
 - Meloidogyne* 229–231, **230**
 - Nacobbus aberrans* 231–233, **232**
 - Paratrichodorus* 235
 - Pratylenchus* 234–235
 - penetrans* 234
 - seed 227, 229, 230, 232
 - tobacco rattle virus (TRV) 386
 - Trichodorus* 235
 - yield losses 227–228, 233, 234
- potato cyst nematodes 5, 9
 - see also* *Globodera*
- potato virus 235
- potato virus Y (PVY) 692, 702
- practical agriculture 1–3
- Pratylenchus* 4, 6, 35, 171, 176–177, 331
 - goodeyi* 35, 622, 625, 626, 627, 637, 640
 - loosi* 587–588, **587**, 588, 589, 590
 - Bacillus subtilis* 606
 - potash 601, **601**, 607
 - Pseudomonas fluorescens* 606
 - tea 585–592, **587, 588**, 604, **604**
- panamaensis*
 - coffea arabica* 557, **558**, 562, **562**
 - coffee 557
 - temperature 560
- penetrans* 178, 195, 703
 - carrot 383, **383**, 384
 - date palm 524
 - olive 481
 - potato 234
 - tea 593
 - tobacco 696, 699

- thornei* 177, 179, 303, 801
 chickpea 303
 maize 195
 wheat 177, 178, **178**, 179
- zeae* 147, 195–196
 cassava 256
Meloidogyne 661, 667
 sugarcane 660–662, 663, 667, 668, 670, 671, 674
- banana 621, 625–627, 644
 biological control 179–180
 biology and life cycle 147, 177, 189–190, 559
brachyurus 424, 425, 426, 559, 560, 561, 724–725
 citrus 461
 cocoa 568
 coffee 556, 557
 cotton 745–746
 cowpea 312
 crop rotation 327
 damage 423–424, **423**, 723–724
 groundnut 423, **423**
 maize 195
Meloidogyne incognita 740–741
 nematocides 426
 peanut 423–426
 pineapple 723–726, **724**, **725**, **726**
Sclerotium rolfsii 425
 sugarcane 660, 662
 sweet potato 239
 tea 592–593
- broad bean 299
 cassava 256–257
 cereal 176–180
 chemical control 179
 chickpea 303–304
 citrus 460–461
coffea 35, **36**, 265, 266, 558, 559–560, 627, 631
 banana 625, 637, 644
canephora 540, **541**, 557, **559**
 carbosulfan 561
 cassava 256
 citrus 459, 460–461, **460**
 cocoa 568
 coffee 556, 557, 560, 562
 crop rotation 275
 economic importance 265–266
 global distribution 625, **626**
 hosts 265, 561, 627
 management 266
 nematocides 561
 oxamyl 266
 physical control 266
 plantain 625, 627, 632
 rhizobacteria 561
 sweet potato 239
 taro 274–275
 turmeric 771
 yam 260, 264–266
- coffee 556–562
 cotton 745–746
crenatus 177
 crop rotation 179
 cultural practices 179
 damage 177, 178–179, 179, 190–191, 557, 559, 626
 economic importance 147, 178, 190–191
 environmental factors 177, 560–561, 660–661
 ginger 769
 grape 486
 haricot bean 315
 hosts 147, 148, 561, 699
indicus 147
jaehni 461
 maize 189–191, **190**, 194
 management 147, 178–180, 303, 327, 561–562
 molecular identification and quantification 178
neglectus 177, 178, 303, 304
 nematocides 191
parazeae 660
 pathotypes 177–178
 peach 26
 peanut 425
 pineapple 723–726
 pistachio 485
 potato 234–235
pseudopratis 256
 resistance 179, 562, 565, 644
 rice 146–147
scribneri 484
 soil temperature 560
 sorghum 196–197
 soybean 326–327, **327**
sudanensis 271
 sugarcane 660–662, 668, 674, **675**, 679
 sweet potato 239–240
 symptoms 147
 tannins 277
 temperature 189
 tobacco 689, 693–694, **694**
 vegetables 383–384
vulnus 461, 477, 481
 yield losses 190–191
- pre-plant management 811–821
 processing 98–99, 346
 protein 290, 321, 411
Pseudomonas fluorescens 606, 760
 publications 4, **4**
 pulses 290
Punctodera chalconensis 192, 194
Purpureocillium lilacinum 256, 372, 372–373, 640, 730, 823
Pyricularia oryzae 124, 127
Pythium graminicola 662, 663

- quantitative trait loci (QTL) 170, 825
 quarantine 6, 14, 128, 358–359, 796, 798, 799
- rabbing 700
- radish 365
- Radopholus* 4, 7, 38–39, **39**, 358, 457–460
 arabocoffeae 563, **563**
 captan 459
 similis 38, **39**, 457, 624–625, 636–637
 arecanut 525–527
 arecanut palm 514, **514**
 avocado 477, 478
 bacteria 760
 banana 518, 622–625, **624**, 631, 632, 636–637, 641, **642**, 644, 803, **803**, 819, **820**, **821**
 betel vine 774
 biology and life cycle 457–458, 515, 525–526, 596, 623–624, 756, 758
 biotypes 459, 624–625
 black pepper 756–761
 broad bean 299
 citrus 457–460, **457**
 coconut 514–518, 519
 coffee 563
 cultural practices 632–633
 damage 525, 596, 623
 DBCP 518
 diagnosis 460, 518, 527
 disease complexes 459, 516
 economic importance 516–517, 526
 environmental factors 458–459, 597
 Fusarium 758
 giant swamp taro 277, **277**
 ginger 768–769
 global distribution 622, **623**
 herbicides 459
 hosts 458, 516, 597–598, 625, 758
 hot water treatment 527–528
 management 459–460, 517–518, 526–527, 632–641
 marotti oil cakes 527
 neem oil cake 526, 527
 nematicides 459, 639
 organic amendments 526–527
 oxamyl 459
 palms 525, 525
 Pasteuria 517
 resistance 518, 526, 635–636, 644
 survival and dissemination 458, 515, 597, 625
 tea 596–598, **596**, **597**
 temperature 458
 turmeric 771
 yam 271
 yield losses 459
- vangundyi* **39**
 vegetables 383
- Ralstonia solanacearum* 356
- random amplified polymorphic deoxyribonucleic acid (RAPD) 106, **110**, 170, 237, 419
- rDNA 170
- read leaf disease 725
- real-time PCR 111–112, **111**
- RealTrichoderm 375
- red palm weevil 519
- red ring disease 513–514, 522, 522–523, 528
 coconut 11, 505–508, **507**, 512, 513–514
 date palm 524
 oil palm 519–520, **520**, 521–523
- reinfestation 603
- remote sensing 812–813
- reniform nematodes
 diagnosis 331
 see also Rotylenchus reniformis
- reproduction and development 23–24
- research 7, 13
 articles 17
 institutions 12–13, 176
- resistance 7, 168, 199, 329, 354
 cultivars 6
 host 9, 10–11, 227, 824–827
 screening 796
- restriction fragment length polymorphism (RFLP) 170, 419
- reverse dot-blot hybridization 105–106, **109**
- rhabditid nematodes 69, 78
- Rhinoceros beetle 519
- Rhizobacteria 80, 291, 374, 544, 561
- Rhizobacteria solani* 425, 545
- Rhizoctonia* 356
 bataticola 310
 solani 356
- rice 6, 24, 38, 120–162, 430
 Aphelenchoides besseyi 125–129, **126**
 Crictonema 148
 Crictonemoides 148
 crop rotation 124, 143–144, 147
 cultivars 124–125, 144, 147, 149
 Ditylenchus angustus 121–125, **122**, **123**, 149
 flooding 135, 136, 359
 foliar parasites 120, 121–129
 Heterodera 144–146, **145**
 Hirschmanniella 133, 139–144, **141**, 149
 Hoplolaimus 148
 hot water treatment 128, 129
 integrated nematode management 139
 Longidorus 148–149
 management 135–139
 Meloidogynae 129–139, **131**
 graminicola 129–130, **129**, **130**, **131**, 132, **132**, 133, **135**, 136, 138, 139, **140**, 149

- incognita* 135
triticoryzae 130, 133, 139
 nematicides 149
 nursery beds 139
Paralongidorus 148–149
 planting date 822
Pratylenchus 146–147
 resistance 124–125
 root
 browning 142
 parasites 129–149
 straw 11
 sunn hemp **802**
 yield losses 120, 121, 124, 127, 133–135,
 142–143, 146–149
 see also paddy rice
 rice–wheat cropping system 136, 137, 139
 ring nematodes 387–388
 RNA interference (RNAi) 679–680
 root
 crops 8, 222, 252
 galling rating scheme 376–377, **376**
 parasites, rice 129–149
 rot 8, 26
 root knot disease 322, 323, 349
 root knot nematodes 9, 10, 11, 68, 182–184, 349,
 807–808
 broad bean 298–299
 diagnosis 330, 414
 vegetables 357–358
 see also *Meloidogyne*
 root lesion nematodes 176–180, 303, 383
 see also *Pratylenchus*
 root-rotting fungi 177
 rootstocks 368–369, 829
 roselle 738, 747–748
 rotation *see* crop rotation
Rotylenchulus 4, 25, 46–47, 745
 chickpea 304
 cotton 744–745
 cowpea 311–312
 haricot bean 315
 macrodoratus 492–493
 nematicides 239
 olive 482
 papaya 482, 483
 parvus 239, 744
 pigeonpea 321
 reniformis 46, **47**, 377–378, 599, 722–723
 banana 629–630
 betel vine 774
 biology and life cycle 377, 721–722
 black pepper 762
 broad bean 299
 carbofuran 311
 chickpea 304
 coffee 563–564
 cotton 744–745, **744**, 801
 cowpea 311
 crop rotation 326
 disease cycle and epidemiology 326
 haricot bean 315
 management 378, 745
 mango 480
 Meloidogyne incognita 741
 mung bean 306
 nematicides 326
 paddy rice 304
 palms 527
 papaya 483–484
 passionfruit 491
 pea 318
 pearl millet 198
 pigeonpea 321
 pineapple 721–723, **722**, **819**
 soybean 325–326, **326**
 sweet potato 238, 239, **239**
 tannias 276
 taro 275
 tea 598–599
 tobacco 697
 vegetables 377–378
 similis 591
 sweet potato 238–239
 tea 599
 variabilis 239
 vegetables 377–378
 vulnus 493
 zeae 321
 rRNA genes 101–102
 rye 164, 171, 182, 185, 186, 309

 sabal palm 512
Saccharum spontaneum 674
 sacrophytic bacteria 177
 saffron 595
 sago palm 504
 sampling 65–68, 87–88
 sapodilla 493
 saprophytes 127
Sclerospora graminicola 198
 Sclerotinia blight 420
Sclerotium rolfsii 415, 425
Scutellonema 43
 brachyurus **44**, 698
 bradys 258–264, **257**
 yam 43, 258–264, **258**, **259**
 cavenessi 432
 seasonality 66
 sedentary endoparasites 24, 26
 seed gall nematodes 180–182
 see also *Anguina tritici*
 seed nematodes 27

- seed potatoes 227, 229, 230, 232
seed treatment 14, 330, 796, 817
 cotton 742, 745
 nematicides 371–372
seedbeds 363, **364**, 821
seedlings 349, 363, 819
 coffee 547, 564, 569
 Meloidogyne 352
 peanut 426
 soilless systems 363
 tobacco 705
 tomato 374
seeds 10, 199, 234, 279
sensors, hyperspectral 812, **813**
sequence characterized amplified region (SCAR)
 markers 420
sesame 260
 straw mulching 803, **804**
shade trees 589, 594
shea butter tree 46
shot-hole borer 591
sieving 87, 93, 94–95
sigma maturity index 72
simple sequence repeat (SSR) 420
simplified faunal analysis 69, 72
Sinapis sisymbriifolium 228
single nucleotide polymorphism (SNP) 825
smallholder farms 1, 3, 5, 8, 10, 11, 566, 816
sodium tetrathiocarbonate 488
soft rot 260, 265
soil 24, 62, 63, 74, 78, 374, 805
 amendments 138, 374, 386, 605, 607,
 729, 743
 bioassays 422
 biological diversity 330, 679
 carbon 69
 decontamination 821, 822
 dry 96
 ecosystems 63, 63, 65
 extraction from 93–96
 food web 69, 74, 76, **76**, 82
 indices 68, 74–76, 75
 metabolic footprints 76, **77**
 soybean 79, **80**
 fumigation 256, 257, 348, 349,
 800, **819**
 health 62–63, 64, 679
 agroecosystems 77–82
 heating 360–361
 management 62–63, 607
 moisture 415
 nitrogen 145
 organic matter 667–668
 pH 354
 puddling 136
 sampling 229, 607–608
 solarization 138, 149, 238, 317, 547
 citrus 455–456
 tea 604
 tobacco 700
 suppressiveness 80, **81**, 82
 soil electrical conductivity (SEC) 812, **814**, **815**
Solanaceae 695
Solanum 222, 223, 368
 rootstocks 829
solarization 374, 811, 813–816, **815**
 Ditylenchus dipsaci 382
 Meloidogyne 360–361, 363
 tomato 361
 see also soil, solarization
sore shin fungus 692
sorghum 133, 182, 196–198, 418, 699
soursop 493
southern blight 415
soybean 6, 46, 179, 290, 298, 314, 315, 320–327,
 329, 371
 brown stem rot 324
 chlorosis 322, 323, **323**, 327
 cultivars 321, 322–323, 324, 825
 fungi 324
 Heterodera glycines 322–325, **324**, 825
 Meloidogyne 322–323
 planting time 325
 root knot disease 322, 323
 root knot galls 322, **322**
 Rotylenchulus reniformis 325–326, **326**
 seed treatment 325, 330
 soil food web 79, **80**
 stunting 327
 sudden death syndrome 324
 temperature 321
 yield losses 322, 323–324, 325
species
 diversity 65, 67, 78
 new 5–6, 7
species-specific PCR assay 170
species-specific primers 178
spices 755–774, 757, 772
spinach 380
spiral nematodes 387
spirotetramat 490
spreading decline, citrus 457
staining 89
starch 252, 450, 504
stem nematodes 184–186, 331
 see also *Ditylenchus*
stem rot 127
sting nematodes see *Belonolaimus*
storage 96, 388
strawberry 82
Streptomyces 176
stress
 abiotic 8, 137
 biotic 8, 137

- structure index (SI) 74, **76**, 78, 79
 stunt nematodes 387–388
 subsistence
 agriculture 329, 348, 796, 808
 crops 796
 farmers 346, 357, 816
 Sugar Yield Decline Joint Venture Programme (SYDJV) 672
 sugarbeet 5, 232, 233, 365, 367, 380, 807
 sugarcane 5, 9, 321, 658, 658–686, 659, 728
 abiotic soil factors 665–667
 bacteria 678
 biotic factors 667–668
 chlorosis 665
 control measures 672–676
 crop rotation 672–673, 679
 cultivars 658, 661, 674, 675, **675**
 cultivation 658–659, **659**, 667, 679
 damage 660, 663–665, 664
 diagnosis 676–677
 disease complexes 661, 662–663, 670–672
 fallowing 672–673, 673, 679
 fungi 678
 green manure 673
 Helicotylenchus dihystra 667, 670
 Hoplolaimus 667
 intercropping 672–673, 679
 Meloidogyne 662–663, **662**, 664, 665, **666**, 668, 674
 nematicides 665, **666**, 670, 670, 673, 676, 677, 678, **678**, 679, 817
 nematodes
 communities 663–668, 670–672
 numbers 668, **669**
 nematode–sugarcane interaction 668–672
 organic amendments 673–674
 Pachymetra 670, 671, 671
 Paratrichodorus 668, 670
 Pratylenchus 660–662, 668, 679
 zeae 660–662, 663, 667, 668, 670, 671, 674
 ratoon cane 670, **671**
 reduced growth 661, 661
 residue 673–674, 679
 root biomass 663, **663**
 shoot count and height 665, **665**
 tolerance 674–675
 Trichodorus 668
 Tylenchorhynchus 679
 weevils 510, 523
 wilt 661
 Xiphinema 668, 670
 yields 668, 672, 673
 losses 661, 663, 676–677
 sunn hemp 79, 309, 366, 430, 699, 705, 728, **802**, 809, 810
 survival 26–27, 808
 SUSFARMS 678
 suspensions, examination 96–98, **97**
 sweet marjoram 490
 sweet potato 5, 222, 231, 235, 235–240
 Ditylenchus 240
 Fusarium oxysporum 240
 Meloidogyne 235–238, **236**
 Pratylenchus 239–240
 Rotylenchulus 238–239, **239**
 yield losses 237, 238, 239
Synedrella 262
 systemic acquired resistance (SAR) 704

Tagetes 366, 379
 tamarind 493
 tannia 252, 276, 277
 taro 5, 252, 271–277, **274**, 800, **800**
 see also giant swamp taro; giant taro
 taxa richness, tomato-maize rotation 78, **78**
 taxonomy 7, 14
 tea 584–616
 biological control 605–606
 botanicals 607
 Brassica 604
 chemical control 603–605
 cover crops 601–602
 cultivars 594, 595, 596, 603, 605, 607, 608
 cultural methods 600–603
 damage 587, **587**, 589, 591, 594, 595, 596, 599
 diagnosis 607–608
 disease complexes 591
 fertilizers 601, **601**, 607
 fumigants 604
 fungi 591
 grafting 603
 green manure 589
 Helicotylenchus 599
 Hemicriconemoides kanayaensis 598
 Hoplolaimus 599
 integrated nematode management (INM) 600
 integrated pest management (IPM) 600
 intercropping 599
 irrigation 602
 management 600–607
 Meloidogyne 593–596, **595**
 mustard oil 600
 neem 600
 nematicides 604, 605
 nematode
 antagonists 605, 606
 species 584–585, 586
 nematode-trapping fungi 605
 oil cakes 601
 organic 600–601, 606–607
 Paratylenchus curvatus 599

- tea (*continued*)
- Pasteuria penetrans* 605
 - physical control 603
 - Pratylenchus* 585–592, 592–593, **587**, **588**, 604, **604**
 - Radopholus similis* 596–598, **596**, **597**
 - Rotylenchulus* 591, 598–599
 - Rotylenchus* 599
 - shade trees 589, 594
 - soft root rot disease 591, 592
 - soil sampling 607–608
 - soil solarization 604
 - Xiphinema* 599–600
 - yield losses 584, 591, 592, 598
- temperature 26, 667
- soil 24
- terbufos 371
- threats, emerging 5–6
- tillage 77–82, 194, 419, 672, 796, 801–802
- Tithonia diversifolia* 256
- tobacco 428, 687–716, 802
- Aphelenchoides ritzemabosi* 697
 - better breeding lines 701
 - biological control 704–705
 - brown spot 692
 - chemical control 703–704
 - chlorosis 690, **691**
 - crop rotation 698–699, 707
 - cultivars 692, 694, 700–703, 705–706, 707
 - cultural control 698–700
 - disease complexes 692–693
 - Ditylenchus* 697
 - fallowing 699
 - flooding 700
 - fumigation 703, 704
 - Globodera* 694–696, **695**
 - Heterodera* 697
 - management 698–706
 - Meloidogyne* 688–693, 689, **690**, **691**, 703, 827
 - mosaic virus (TMV) 692
 - nematicides 703–704, 707
 - non-fumigant nematicides 704
 - planting date 822
 - ploughing 698
 - Pratylenchus* 689, 693–694, **694**, 696, 699
 - production 687, 698–699, 707
 - rattle virus (TRV) 386, 698
 - resistance 700–703
 - ringspot virus 698
 - Rotylenchulus reniformis* 697
 - Scutellonema brachyurum* 698
 - seedlings 705
 - soil 687, 700
 - stem break 697
 - trap crops 699–700
 - Tylenchorhynchus* 697–698
 - yield losses 690, 693, 694, 696, 697
- tolerance 26, 824–827, 824
- tomato 227, 231, 350, 365, 373, 385, 387, 823
- BioNem 373
 - Crotalaria longirostrata* 366
 - cultivars 367, 368–369, 826, **828**
 - fenamiphos 371
 - Fusarium* 355–356, 375
 - wilt 374
 - Globodera* 379–380
 - marigold 366
 - Meloidogyne* 361, **362**
 - arenaria* 354, 359
 - enterolobii* 350, **350**
 - incognita* 130, 352, **352**, **353**, 354, 366, 368, 373, 375
 - nematophagous fungi 375
 - oxamyl 371
 - Pasteuria* 373–374
 - Rhizobacteria 374
 - Rhizoctania* 356
 - rootstocks 368–369, 829
 - seedbeds 363
 - seedlings 374
 - solarization 361
 - spotted wilt virus (TSWV) 420
 - Tagetes erecta* 379
 - Trichoderma asperellum* 375
 - yield losses 357, 358, 373, 377–378, 824, 829
- tomato–maize rotation 78, **78**
- totally impermeable films (TIF) 488
- toxins 26
- transgenic crops 11
- transportation 346, 388
- trap crops 228–229, 233, 365, 699–700, 808–809, 809, 811
- trapping fungi 184
- tree crops 826, 827
- Trichoderma* 128, 311, 360, 373, 375
- Trichodoridae, morphology **21**, 23
- Trichodorus* 58–59, **58**, 235, 385–386, 463, 480, 668
- viruliferus* **58**
- triticale 164, 171, 179
- trophic groups 64, 64, 65
- Trophotylenchulus* 564, 763
- tropical agriculture
- future developments 15
 - research 13
- tropical forages, peanut 417
- tropical–temperate interaction 4–5
- tropics 1, **2**, 3, 5, 14
- tuber crops 8, 222, 252, 349, 352
- turmeric 769–772
- tylenchids, feeding on root tissue 24, **25**
- Tylenchina, morphology 20
- Tylenchorhynchidae, broad bean 299

- Tylenchorhynchus* 33–35, **34**, 387, 430–431, 434
 sorghum 197
 sugarcane 679
 tobacco 697–698
- Tylenchs, morphology 21–22, **21**
- Tylenchulus* 25, 54–56
semipenetrans **55**, 446, 447, **450**
 biological control 456
 biology and ecology 447–451
 biotypes 451–452, 455
 citrus 447–456, **448**
 crop management 455
 disease interactions 452
 exclusion 454–455
 Florida nursery certification programme 454–455
Fusarium solani 452
 grape 486, 487, 488
 management 454–456
 mangosteen 493
 nematocides 454
 olive 482
Pasteuria 456
 persimmon 484
Phytophthora nicotianae 452, 454, 455–456
 rootstock resistance 451–452
 salinity 450–451
 sampling and extraction 453
 soil moisture 448–449
 soil water potential 448, **449**
 temperature 448–449
 yield losses 453–454
- ufra 121, **123**, 124
- universal primers 104
- universities, nematology 12
- upland cotton 738
- urd 327–328
- urea fertilizer 195
- vegetables 346–410
Belonolaimus 384–385
 biological control 388–389
 biological enhancement 374
Cactodera 380
 cultivation techniques 348
 cyst nematodes 379–387
Ditylenchus 380–383
 exports 348–349
 fresh 346, 348, 348
 fumigants 358
Globodera 379–387
Heterodera 380
Longidorus 386–387
 management 375–376
Meloidogyne 349–377, 351
 mycorrhizal inoculum 374–375
Nacobbus aberrans 379
 nematocides 358, 388
 nutrition 346
 paddy rice 359, **360**
Paralongidorus 386–387
Paratrichodorus 385–386
 peri-urban production 348
Pratylenchus 383–384
 production 346, 347, 348, 349, 358, 362, 388
Radopholus 383
 resistance 368
 root knot nematode management 357–358
Rotylenchulus 377–378
 stunt nematodes 387–388
Trichodorus 385–386
Xiphinema 386–387
 yield losses 349
- vegetarianism 321
- velvet bean 673, 810
- Verticillium dahliae* 227, 481
- vine crops 486–492, 827
- virtual hubs 13
- virulence 228, 826
- VOTIVO 823
- water 24, 27, 62, 124
- water hyacinth 600
- watermelon 428
- watery rot 260
- wax moth larvae 82
- weeds
 fallow 806
Meloidogyne 369, 370
- weevils 510, 621, 631
 palm 29, 504, 506, 508–512, **508**, **509**, 513, 521, 523, 524, 528
 red palm 519
- wet rot 260, 261, 265
- wheat 8, 163–186, 183
Anguina tritici 180, **180**, 181, **181**, 182, **182**
 breeding programmes 179
 cultivars 179
 durum 171, 179
Heterodera avenae 167, **167**, **168**, 171
Meloidogyne 136, 183, **183**, 184
Pratylenchus 177, 178, **178**, 179
 resistance 171, 172, 173, 174, 175
 seed hygiene 182
 yield losses 178, 183
- wheat–rice cropping system 136, 137, 139
- white tip 125–126, **125**
- wild grass 171
- wild palm 522

- wildfire disease 702
wilt 330, 670
winged bean 328
witches' broom 566, 569, 764
- Xanthomonas* bacterial wilt (BXW) 632, 644
Xiphinema 23, 25–26, 56, 57, **57**, 198
 brevicolle 477, 479, 480
 citrus 463
 cotton 747
 grape 486, 488
 ifacolum 484
 index 478
 maize 187, **189**
 olive 481
 sugarcane 668, 670
 tea 599–600
 vegetables 386–387
- yam 5, 11, 252, 257–271
 Aphelenchoides besseyi 271
 Chromolaena 262
 crazy root symptoms 267, **267**
 crop rotation 270
 dry rot disease 258–259, **259**, 260, 261, 265, 266
 Eupatorium 262
 intercropping 261, 270
 Meloidogyne 266–271, **267**, **269**
 neem 262
 Paratrachodorus porosus 271
 pesticides 264
 Pratylenchus 260, 264–266, 271
 Radopholus similis 271
 Scutellonema bradys 43, 258–264, **258**, **259**
 seed tubers 262, **262**
 Synedrella 262
 tuber quality 269
 yield losses 264, 265–266
- yellow ear rot 180–181, **181**, 182
yields 1, 9, 81, 163, 797, 805, 824, 825
 banana 618
 cucumber 369
 legumes 290–291, 292
 losses 3, 7, 8, 8, 10, 329, 825
 banana 632, 644
 barley 183
 Belonolaimus longicaudatus 385
 cassava 255, 256–257
 cereal 199
 chickpea 300, 303
 citrus 452, 453–454, 459
 cocoa 566, 567, 569
 coffee 545–546, 555–556, 557
 cotton 743
 cowpea 307
 grape 486, 487
 haricot bean 313, 314, 315
 Heterodera 170
 lentil 305
 maize 190–191, 192
 Meloidogyne 357, 416, 417, 663
 papaya 483
 pea 317
 peanut 414, 416, 417, 423, 424, 425, 428, 430
 pigeonpea 320
 pineapple 718, 719, 721, 723, 725, 731
 plantain 632
 potato 227–228, 233, 234
 Pratylenchus 190–191
 Radopholus similis 459
 rice 120, 121, 124, 127, 133–135, 142–143, 146, 147, 148, 149
 soybean 322, 323–324, 325
 studies 14
 sugarcane 661, 663, 676–677
 taro 272
 tea 591, 592, 598
 tobacco 690, 693, 694, 696, 697
 tomato 357, 358, 377–378
 Tylenchulus semipenetrans 453–454
 vegetables 349
 Verticillium dahliae 227
 wheat 178, 183
 yam 265–266
 sugarcane 668, 672, 673
 tea 584
 tomato 373, 824, 829
 tropics 5
 yam 264
- zinc 165



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